An investigation into the biosynthesis of cuticular hydrocarbons in British ants using isotopically labelled diets and gas chromatography - mass spectrometry

Susan Jayne Shemilt

Thesis submitted for the degree of Doctor of Philosophy

October 2021

Keele University

Abstract

Ants are one of only four groups which can be classed as being truly social, that is they demonstrate altruistic behaviours towards nestmates. However in order to do this there must be a complex nestmate recognition signal at work. This signal is generally poorly understood, however it is accepted that it makes up a small part of the overall chemical profile present on the cuticle of the ant. This cuticle is coated with a variety of long chain hydrocarbons; the biosynthesis of which is a complex, multi-step process which is generally not well studied in social species. Many of the biosynthetic pathways already discovered have been done so using other insect species which are traditionally easier to work with. Therefore the main aim of this study was to test whether these biosynthetic pathways were also applicable to our studied British ant species.

In order to aid the elucidation of the biosynthetic routes, simple feeding experiments were devised. These experiments involved feeding a variety of species of common Britsh ants artificial diets. The species chosen for these experiments belonged to the subfamilies Formicinae and Myrmicinae and the diets were modified by adding an isotopically labelled substrate. The experiments of this type explored the incorporation of these labels into the final biosynthesised hydrocarbon product. Successful incorporation suggested that the substrate was a biologically important molecule to the biosynthetic process; either used as a precursor or later in the biosynthetic process. The results presented within this thesis showed that the incorporation of these labelled substrates depended on a number of factors. The amount of incorporation appeared to vary by species, substrate and label type, as well as possible environmental and instrumental factors such as ant health and instrument sensitivity.

It was generally found that the labels based on deuterium atoms were less reliable as their more labile nature meant that any trends were less discernible. More reliable and insightful results were gained when using carbon-13 labels. The incorporation appeared

to vary with some species showing different responses to others, however the reasons behind this difference are unknown. It could be down to subtle differences between the biosynthetic pathways or a previously unconsidered factor could be important, such as the health of the colony or rate of hydrocarbon production.

The main challenge of this work was sourcing both the correct species of ant, and labelled substrates that were cost effective to use. Custom organic synthesis was used in order to produce a wide range of labelled fatty acids, however ultimately experimental design was dependant on commercial substrate availability.

The results shown within this thesis indicate that there is a lot of unknown knowledge surrounding biosynthetic processes in ants and that models gained from other species cannot necessarily be applied to ants. However, it is also clear that there are many possible dependant factors influencing this work and that far more research needs to be carried out in order to understand the underpinning biosynthetic processes more clearly.

Contents

List of Tables

List of Figures

Abbreviations

CHC	Cuticular hydrocarbon
GC-MS	Gas Chromatography-Mass Spectrometry
NMR	Nuclear Magnetic Resonance
CoA	Coenzyme A
K_a	Acid dissociation constant
SACP	Sulfur acyl carrier protein
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced form)
GC	Gas Chromatography
SPME	Solid Phase Micro Extraction
GC-FID	Gas Chromatography-Flame Ionisation Detector
DMDS	Dimethyl Disulfide
HETP	Height Equivalent to Theoretical Plate
FID	Flame Ionisation Detector
CI	Chemical Ionisation
EI	Electron Ionisation
SIM	Selective Ion Monitoring
MALDI	Matrix-assisted Laser Desorption/Ionisation
ToF	Time of Flight
MALDI-ToF	Matrix-assisted Laser Desorption/Ionisation-Time of Flight
DART	Direct Analysis in Real Time
DART-MS	Direct Analysis in Real Time-Mass Spectrometry

Acknowledgements

The story of this PhD began well over 10 years ago when my PhD supervisor informed me of a upcoming research assistant job within the chemical ecology group after I had graduated my undergraduate degree. Whilst I hadn't seen myself going down this route, in all honesty I had no real idea what I wanted to do in terms of career, this seemed like something that I would enjoy and a fantastic opportunity. Fast-forward several years when the project came to an end and I had to say goodbye to my research, luckily for me I was able to stay nearby, undertaking a teaching role within the Forensic Science department. Later, when the opportunity arose for me to do a fully-funded PhD in chemical ecology, I knew it was something that I wanted to do. For this I have to thank the generous funding sources of this project; The Natural Environment Research Council as well as various sources within Keele, particularly ACORN funding, without their generosity this project would not be possible.

My supervisor Falko Drijfhout is someone that I have had the pleasure of knowing in many different capacities; from a lecturer of mine to my PhD supervisor, and for many years, also a trusted colleague. In all capacities I have been lucky enough to have had his guidance, help and support, not to mention the fantastic summer barbeques! So thank you Falko for thinking of me back in the summer of 2008, I will forever be truly grateful to you for starting me off on this journey.

Ultimately there are people who deserve a very special mention. Firstly Merijn Vriends, an overseas student who joined us for the best part of a year. Merijn was quiet and unassuming, but brilliant at organic chemistry, and responsible for synthesising all those compounds used within the fatty acids chapter. Thank you Merijn, for working so hard and not giving up, especially when the synthesis didn't quite work as it should. I would like to take this opportunity to thank two of my very best of friends. These two started as students that I was responsible for teaching and went on to become lifelong

friends. It is because of them that this thesis has apostrophes in the right place and that et al. has a full stop! So thank you to Kate and Amy, I don't know what I do to deserve the two of you but I'm so glad that you keep me around!

I would like to take this opportunity to mention Stephen Martin, I'm not sure I have ever met someone so enthusiastic about research! Your passion inspired me so many years ago to undertake a PhD and more recently your digging skills have kept me suitably stocked with ants so thank you.

I would also like to thank my family, it's often the case that with PhD's, no-one is really quite sure what you spend your days doing, nor do they truly understand the problems you try to explain, nor the stresses. But despite this, a special thanks to my wonderful Mum for your love and support, and for keeping me fed, watered and generally happy, for the whole of my time in education. I would also of course like to pay a very special thanks to my wonderful partner Andy; you have done more for me than you will ever know, so thank you.

Finally a few more special mentions. Firstly a special thanks for Jane; who offered to proof read my final thesis many years ago, I promised I would hold you to that offer and I did! Thank you for doing such a great job proof reading and helping to make sure that I fully explained all aspects of this work - my thesis is stronger for it, so again, thank you. Finally to all those office members who deserve a special mention; thanks to Richard - a great travel companion and an even better sounding board. For Steve and Swanny - thanks for the laughs and technical expertise. Finally thanks to Dave - from those days sat in front of an HPLC to a really good friend and mentor.

Having to balance a teaching role as well as this PhD has meant that this project has taken me longer to complete, having spent only one year as a full-time student. As a result I have shared an office with lots of other amazing people and I have many

fantastic memories of my time as a student. There really are too many individuals to mention here; but over the years I have seen many students start and finish their PhD. It feels very strange for myself to finally be in this position having watched many others go before me, but now it's my turn...

Chapter 1

Introduction

1.1 Ant Background

Within the invertebrate animal kingdom there are only four classes of eusocial insects; that is, insects which can be classed as being 'truly social'. For this classification three characteristic traits must be present [1–3]. The first is that there must be a clear division of labour. This is achieved using a caste system with non-reproductive workers assisting those that are capable of reproduction. There must also be clear cooperation between workers in terms of caring for and rearing young. Finally there must be overlapping adult generations, allowing for many individuals to contribute to the function of the colony [1–3]. Along with ants, the other eusocial insects are termites, wasps and bees, although it should be noted that not all species of wasps and bees are truly eusocial. Of the four eusocial insects, ants are the most numerous with an estimated 11,700 species formally recognised, a number which is constantly growing as new species are discovered [4]. Ants are extremely successful, they are found in every landmass on Earth, with the exception of Antarctica. They are able to exist in various different types of habitats, with differing temperatures, elevations and ecologies, and as such, different species have diversified to become herbivores, scavengers and predators [5]. Ants account for between 15-25% of the total global animal biomass [5] and belong to the family *Formicidae* with 21 extant subfamilies [4] as shown in Figure 1.1.

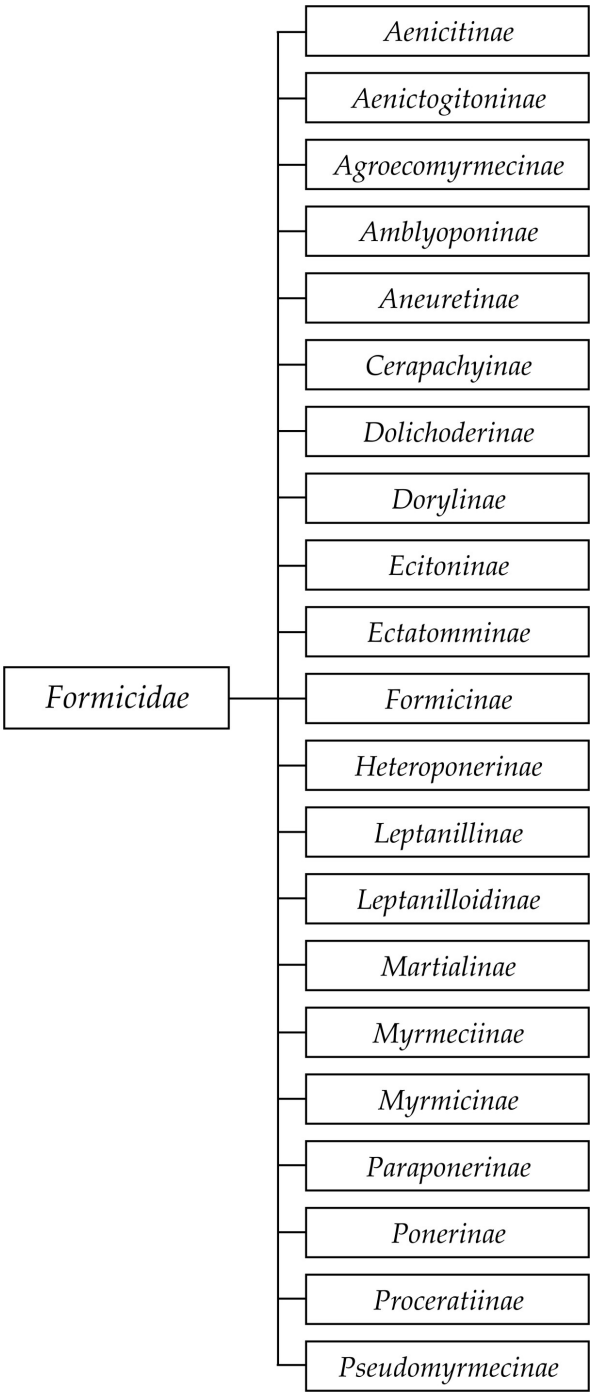


Figure 1.1: The subfamilies of the *Formicidae* family, adapted from [4].

Although globally there are over 11,000 species of ant, only a very small number of these can be found within Britain [4]. The number of species present in Britain varies depending on the literary source. However according to the National Bees, Wasps and Ants Recording Society there are, within four subfamilies, an estimated 51 native species found in Britain with a further three species found only in the Channel Islands.

In addition there are an estimated 13 introduced species found exclusively in artificially heated environments [6]. The small number of ant species found within Britain is generally due to the sun-loving nature of ants and Britain's mostly temperate climate. Due to this, ants tend to be more abundant within the southern parts of the country or within warmer climates. Similarly, ants themselves are only active during the warmer parts of the year; particularly on sunny days [7].

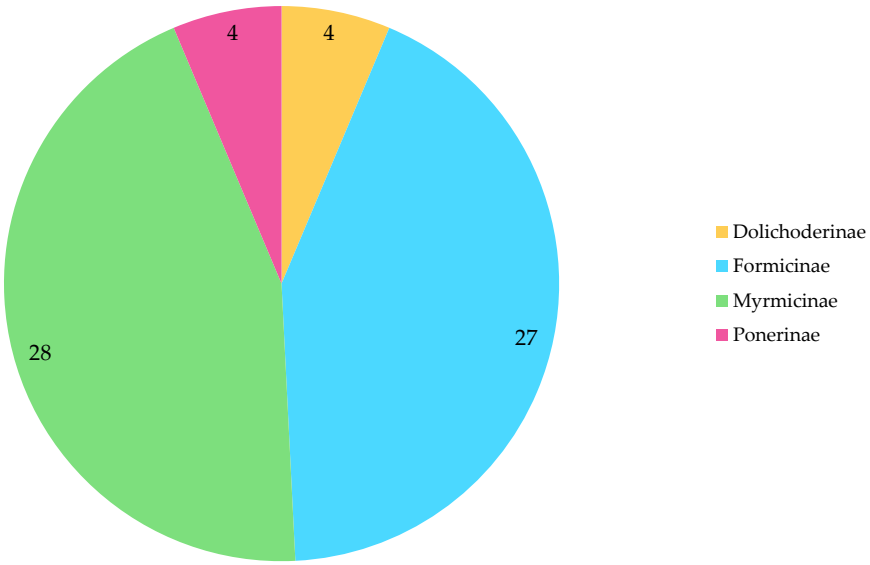


Figure 1.2: A pie chart showing the four subfamilies of ants present in Britain, the number of species belonging to each family is indicated. However information about the species present in Britain varies greatly between sources and so this diagram should be treated as an estimate only. Adapted from [7].

1.1.1 Biology

Although there are many thousands of ant species currently known, all share key characteristics. These characteristics are: the ability to produce both winged and wingless females, a specialisation of the first of the abdominal segments to form one or two nodes, antennae featuring a long first joint and a larger number of smaller joints, and mandibles which meet medially [2]. For a diagram showing these features see Figure 1.3. As part of this cooperative sociality, ants form colonies that vary greatly in size but can consist of a few individuals living in small cavities, up to highly organised

colonies which can contain millions of individuals and occupy large areas of space. In its simplest form a larger nest will contain a single fertile queen; this is known as a monogynous colony. Alternatively there may be multiple fertile queens and many sterile female workers, this is known as a polygynous colony [7]. There may also be males present, however these are short lived and do not undertake social tasks [7]. Instead it is the female workers that undertake tasks within the nest, such as caring for the brood, foraging and general housekeeping duties [8].

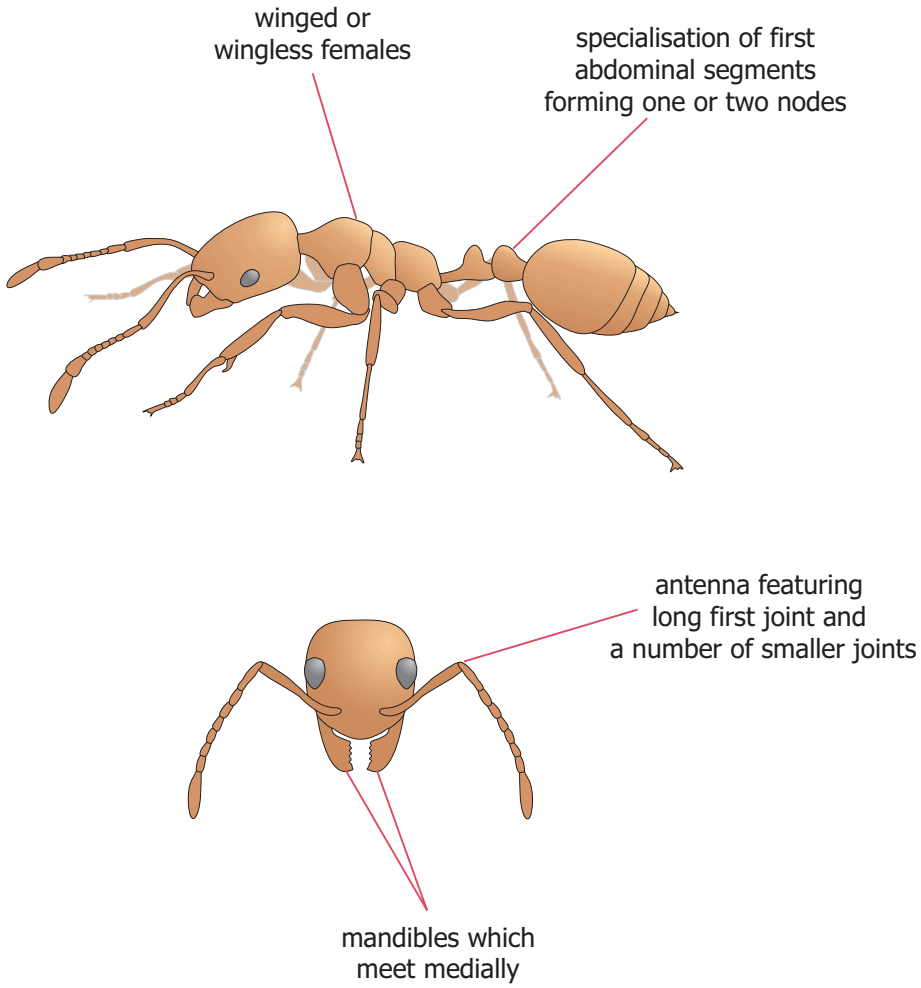


Figure 1.3: A diagram showing the common characteristics of all species of ants.

Ants demonstrate a strong caste based system, which consists of a reproductive queen, or group of queens, which is aided by non-reproductive workers. Within some species

there is also a soldier group, whose role is to protect the colony from attack. Ants demonstrate haplodiploid reproduction; that is the queen governs whether an egg will hatch into a male or a female ant. By releasing stored sperm, haploid eggs will develop into diploid female offspring, i.e. with inherited chromosomes from both mother and father. If however, sperm is not released, the eggs will remain unfertilised and develop into haploid males, with just maternal chromosomes present [1, 2].

1.1.2 Kin recognition

Within all eusocial insects there must be a robust system of kin recognition underpinning behaviour. According to Hamilton's rule [9, 10] recognition of kin should enhance fitness as it allows for individuals to direct altruistic behaviour, such as social feeding, towards close kin, i.e. nestmates. Within an ant colony however, there are generally millions of related, yet sterile workers. Therefore for these individuals, the only way to increase the fitness of the colony as a whole, is by directing altruistic behaviours toward genetic relations. This may suggest that the recognition system is based on genetic cues. However this is considered to be a fundamentally flawed theory as it would lead to nepotism within colonies in which individuals are not all direct relations. For example in polygynous colonies, which have more than one queen, some workers may be unrelated, therefore if a wholly genetic cue did exist, workers would be expected to favour only their related individuals. However evidence to suggest nepotism in polygynous colonies has yet to be conclusively found despite many studies in the area [11–15]. In the study by Ratnieks et al., the authors postulated that such behaviour would result in an inefficient colony due to time wasted determining genetic relatedness, however other reasons have been offered for the lack of nepotism found within polygynous colonies. In a study by Sherman et al. [16] the authors suggest that nepotism is unfavourable due to the high amount of recognition errors. Such errors would lead to altruistic acts being directed to non-kin and thus would reduce the effect of nepotism so that it is no longer a beneficial act. The final explanation for a lack of nepotism is offered by Reeve [17] who suggests that some colony members benefit from

there being no kinship recognition within the colony. It is the lack of evidence for true nepotistic actions within polygynous colonies which has led most researchers to feel that kin recognition is based on the recognition of nestmates rather than true kin [18]. However as the biosynthesis of these chemical nestmate recognition cues is under genetic control [19] there must be some genetic influence over their production. It is therefore now believed that there is contribution from genetically determined biosynthesis and a general mixing of these cues to form an overall colony 'odour' or profile. This is known as the 'gestalt' model, named after the german word for whole, but more recently the word is known to indicate a whole which is worth more than the combined sum of its parts [20, 21].

1.2 Cuticular Hydrocarbons

1.2.1 Background

Within insect species the outermost surface is known as the cuticle and forms part of the insect's exoskeleton [1]. The cuticle is covered in a stable layer of hydrophobic lipids, the chief purpose of which is to prevent desiccation of the insect via evaporative water loss [1, 19, 22, 23]. Within ant species these lipids are normally long chain hydrocarbons i.e. those with a carbon chain length of greater than 20 carbon atoms, up to, typically, 35 carbon atoms [24]. However, high temperature studies utilising new high temperature gas chromatography columns have yielded compounds up to 48 carbons in length [25, 26]. The hydrocarbons found on the cuticle are commonly referred to as cuticular hydrocarbons or CHCs, and are typically straight chain unsaturated alkenes, saturated *n*-alkanes, or mono, di and occasionally trimethyl-branched alkanes [27]. Figure 1.4 shows the basic structures of these compound types. Additional compounds which can also be found on the cuticle include wax esters, fatty acids and sterols [28–30]. The mix of chemical compounds present on the cuticle of insects is commonly referred to as their individual chemical profile, or CHC profile.

Although the chief purpose of these hydrocarbons is to allow the insect to thrive in sometimes hostile terrestrial environments it has become clear that these compounds also serve as signalling cues. The reasons for the wide variety of chemical compounds that may be present on the cuticle are still not fully understood, however some researchers believe that each compound, or mix of compounds, could indicate for a different signal, such as nestmate recognition, task allocation and caste [30, 31].

The blend of hydrocarbons that is found on the cuticle of ants is tightly controlled. Different species use different hydrocarbons within their chemical profiles, and thus these profiles are species specific [32–34]. The CHC profiles of varying species can be readily identified using the analytical chemistry technique gas chromatography-mass

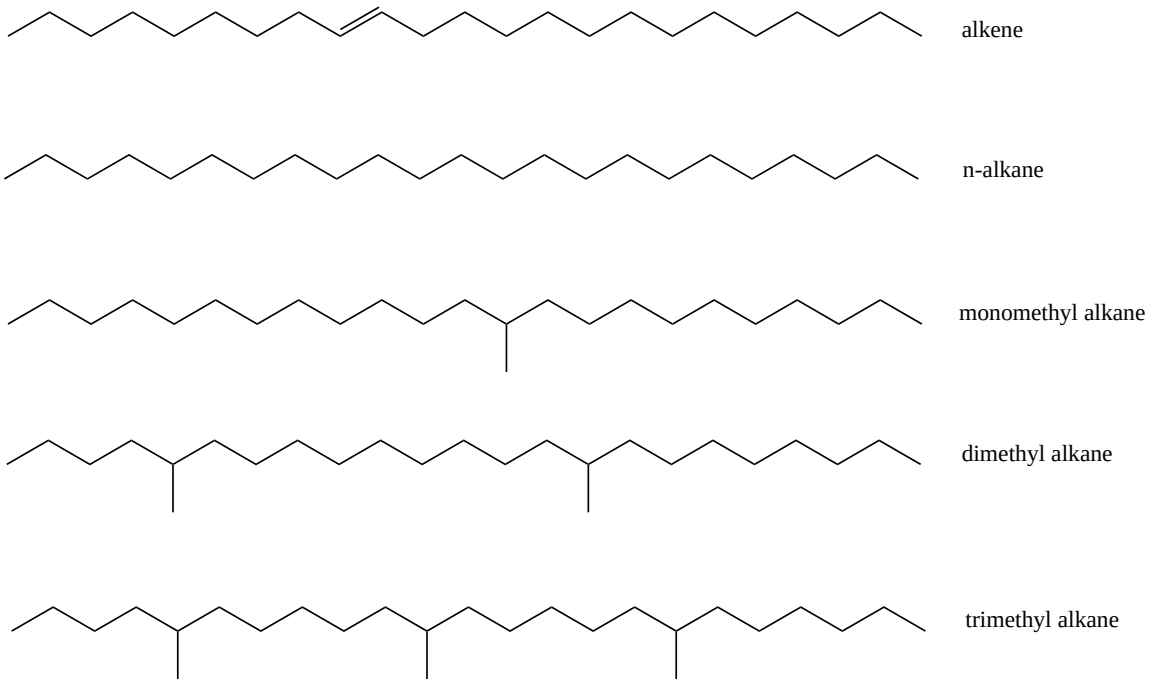


Figure 1.4: Commonly found cuticular hydrocarbons.

spectrometry (GC-MS). Using this technique the chemical profile present on an ant's cuticle can be represented as a chromatogram, an example of which can be seen in Figure 1.5. This Figure shows the chemical profiles (as chromatograms) of three different *Formica* species. As can be seen, these three species, all within the same genus, have totally different CHC profiles. *Formica lugubris* has the most complex profile featuring longer chain hydrocarbons and many branched compounds, including mono, di and trimethyl compounds, whereas *Formica exsecta* features pairs of odd numbered alkenes and alkanes, ranging typically from 23 carbons in length, to 27. Finally *Formica lemani* has the simplest profile of the three with very few compounds and is typically dominated by large amounts of pentacosene.

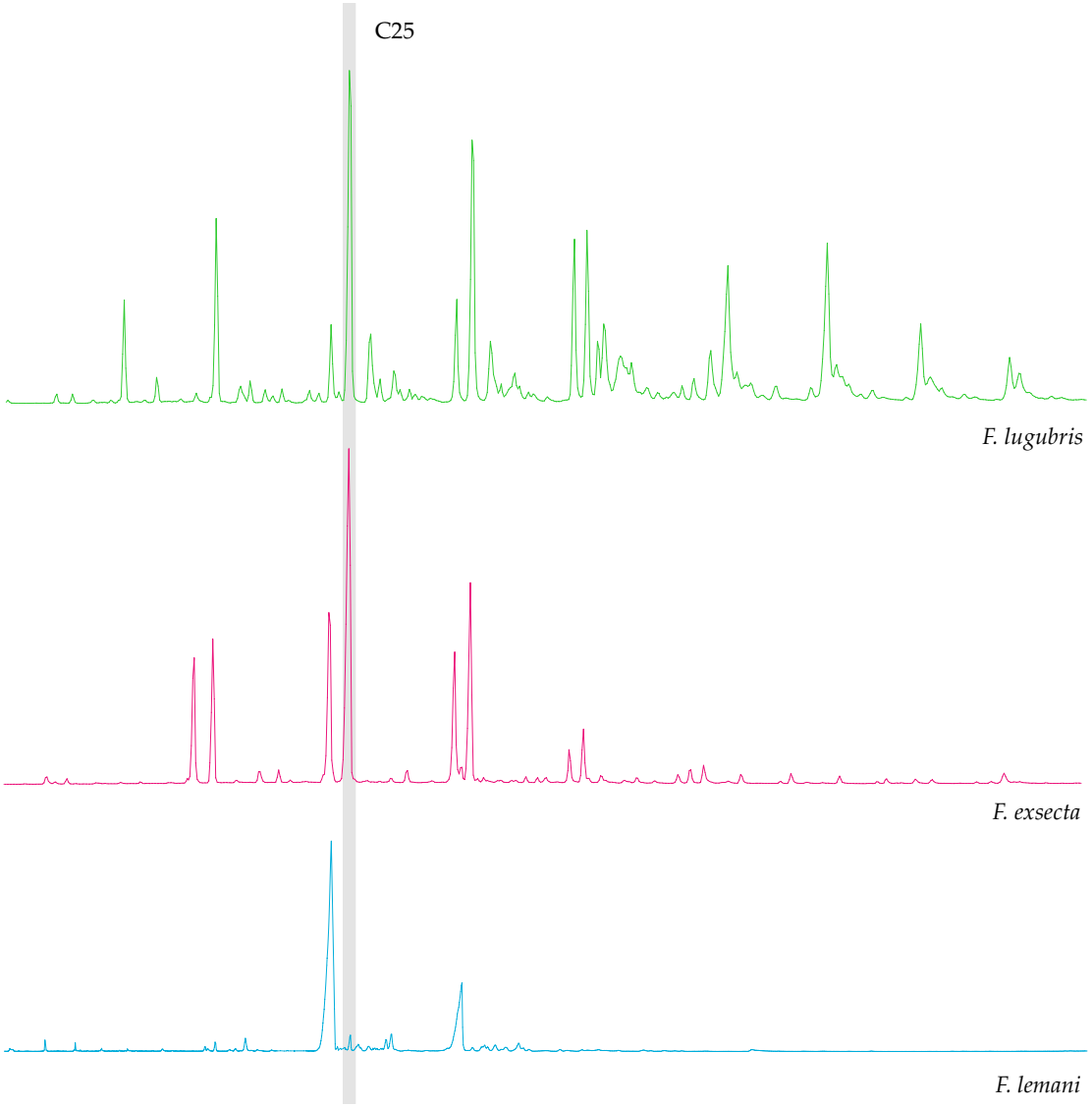


Figure 1.5: Representative chromatograms showing the cuticular hydrocarbon profiles of three *Formica* ant species. The grey bar represents the compound pentacosane, which is common to all three species. For the fully labelled chromatograms of the experimental study species *Formica lugubris* and *Formica lemani* please see Appendix A.

Within each species the ratios of the compounds within the chemical profile varies, it is this varying ratio that encodes the colony identity. Thus when an individual encounters another, it is a simple task for the ant to detect whether the specific ratio of CHC compounds is the same as its own. However research has shown that in some cases it is only certain compounds within the profile that encode for the colony identity. One study which investigated nestmate recognition was that in 2008 by Martin et al. [32].

In this study the cuticular hydrocarbon profiles of ten *Formica exsecta* colonies were analysed using GC-MS. *Formica exsecta* has a very simple CHC profile which, as previously mentioned, consists of just a few alkenes and alkanes and therefore it is the ideal study species as it is very straightforward to analyse. It was found that whilst the alkane component can show variation, the alkenes are much more tightly controlled. The results of this study therefore suggested that the alkenes are responsible for colony recognition within this species. This was later confirmed using bioassays and synthetic mixtures of alkenes [32]. Figure 1.6 shows the previously obtained chemical profile of five workers from a single colony of *Formica exsecta*. This data has been processed from the raw chromatograms and presented as a bar graph. This is a standard simplified representation of a chemical profile and is commonly used because it removes the differences in actual compound amounts and instead represents them as percentages of the total amount of hydrocarbons present. See Figure 1.6 for a visual representation of the colony profile and the variation between workers sharing this profile.

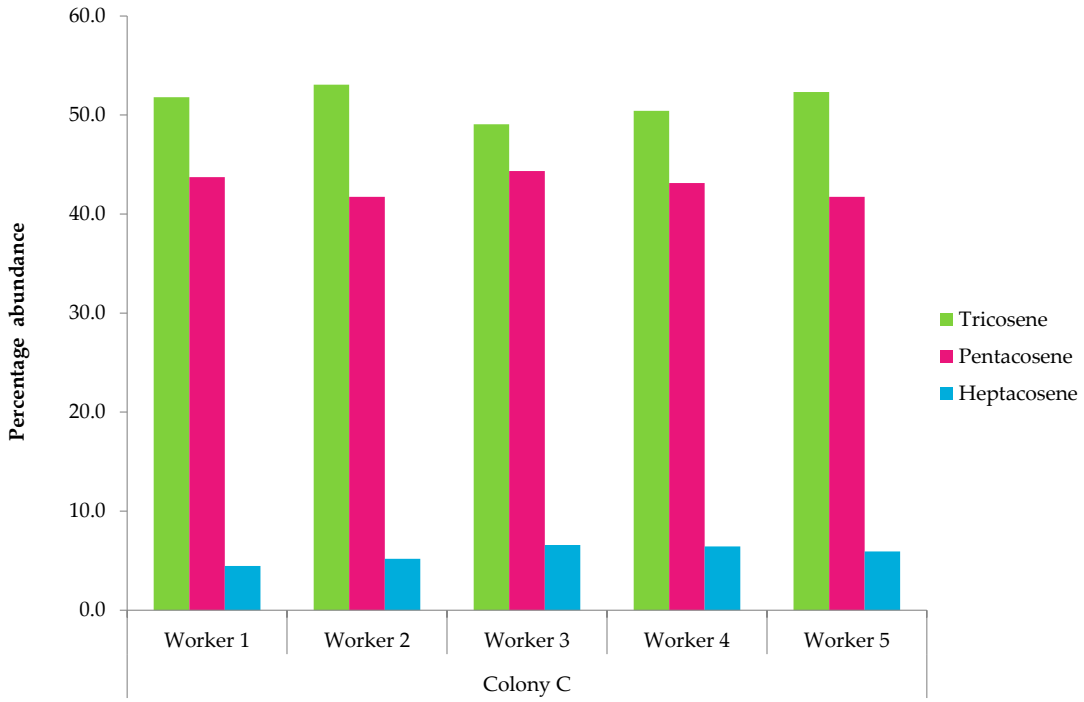


Figure 1.6: Visual representation of the cuticular hydrocarbon profile of *Formica exsecta* showing the variation between worker profiles within a colony. Note that the data shown in this figure is based on real data collected on this species prior to the commencement of this research project.

Figure 1.6 shows how the CHC profile of workers within a colony is tightly controlled and extremely consistent. Although subtle differences can be observed between workers within a colony, the overall profile remains very similar. In contrast, Figure 1.7 shows the profile of four workers from four different colonies and shows how inter-colony variation exists; whilst the compounds within the CHC profile are the same, the ratios of the compounds varies, and thus a unique colony profile is created. Although the differences between colonies can be subtle, ants are able to detect this difference and thus recognise their own nestmates.

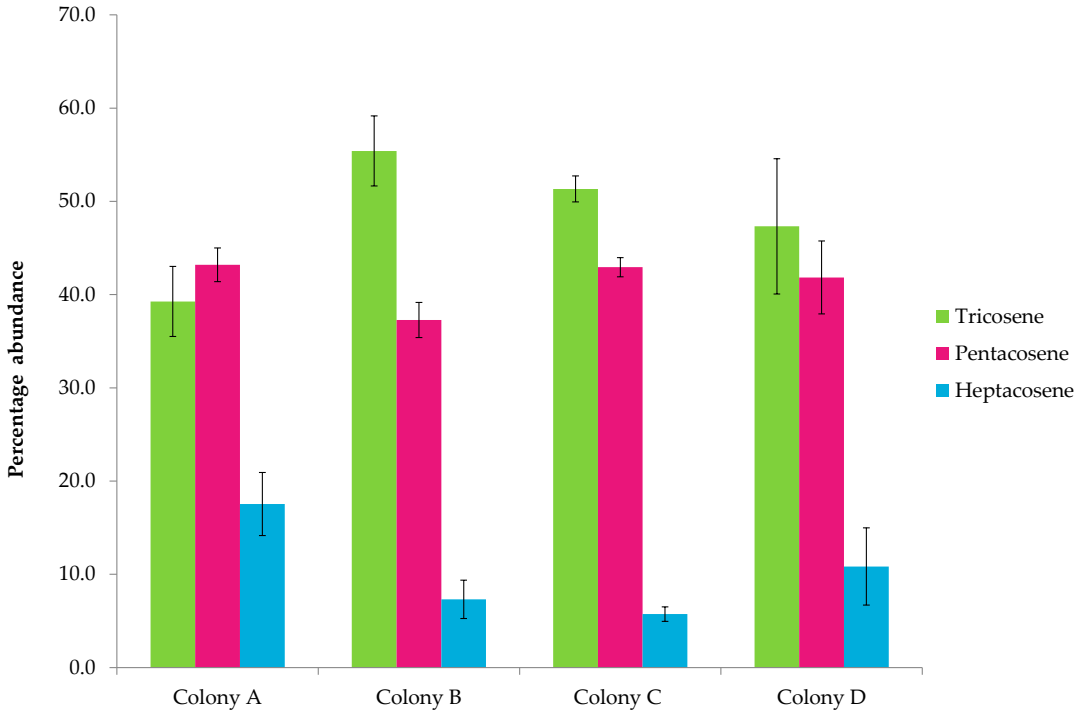


Figure 1.7: Visual representation of the variation in cuticular hydrocarbon profiles between four different *Formica exsecta* colonies. Note that each colony profile is based on the average chemical profile from 5 workers, with error bars indicating +/- two standard errors. Note that the data shown in this figure is based on real data collected on this species prior to the commencement of this research project.

It has long been proven that CHCs exist in species specific mixtures, with some species having very simple profiles with few compounds and others containing many di, tri and even tetramethyl alkanes [27, 30, 35, 36]. Therefore CHC profiles have the potential to be used as powerful identification tools for chemotaxonomic purposes where morphological species identification is difficult and time consuming [33, 34, 37, 38]. One example of the chemotaxonomic potential of cuticular hydrocarbons was demonstrated in the study by Guillem et al. [33]. In this study the author was able to chemically distinguish between two very morphologically similar species; *Myrmica sabuleti* and *M. scabrinodis*. Traditionally these species are identified by studying the shape and size of the antennal lobe at the base of the antenna, [33] however this requires the use of microscopy and a trained eye. The CHC profiles however, whilst similar, showed clear differences; *M. sabuleti* showed greater amounts of 5-methylpentacosane whilst

M. scabrinodis showed greater amounts of 3-methyltricosane.

Considerable research has been done into the physical properties of these hydrocarbons and it has been found that the structure of the hydrocarbons directly affects their physical properties. As expected, longer chain lengths result in a higher melting point, however the presence of a methyl-branch or a double bond within a hydrocarbon can also influence its properties. In a major study by Gibbs [39] the author studied a wide variety of hydrocarbons and their respective melting points. The author found that the addition of a methyl-branch lowered the melting point of the hydrocarbon as the branching interfered with the packing of the hydrocarbon in its crystalline state. This reduced the strength of the intermolecular bonding and hence lowered the melting point.

In an earlier study [28] the effect of methyl-branches on melting point was more comprehensively researched and it was found that the degree to which this melting point was lowered was not uniform, instead it depended on the position that the methyl-branch was inserted. If the methyl-branch was positioned centrally within the molecule, for example as seen in 11-methylpentacosane, the reduction in melting point compared to the straight chain equivalent was considerable at approximately 35 °C. However if the methyl-branch was inserted in a more terminal position, for example as seen in 3-methylpentacosane, the effect was much less, with a gradual transition between these extremes. This suggests that the more central methyl-branches cause a greater disruption to the packing of the hydrocarbons, resulting in a lower melting point. See Figure 1.8.

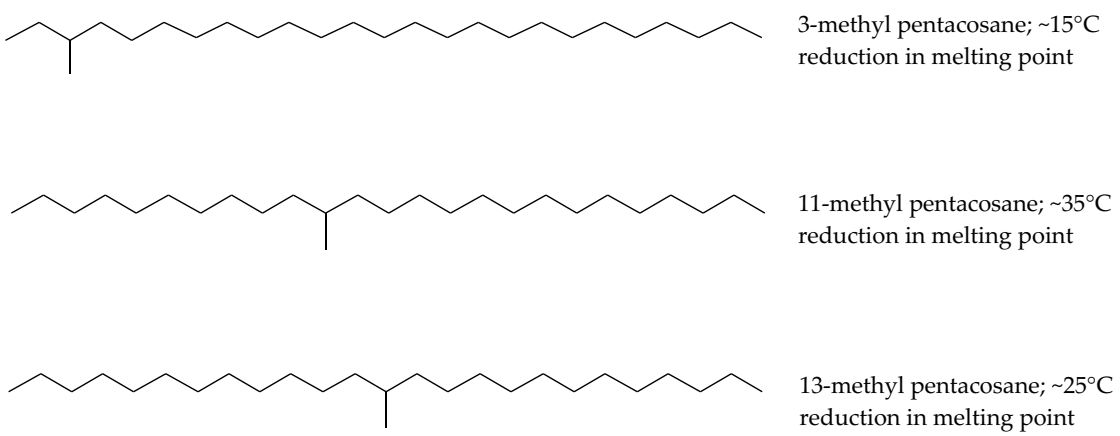


Figure 1.8: The structures of methyl-branched hydrocarbons and the effect of the position of the methyl-branch on melting point.

It is worth mentioning however that the most central position for a methyl-branch within pentacosane would be in the 13 position. For this compound the authors of this study found that there was less of a reduction in the melting point compared to the straight chain alkane by approximately 10 °C. They attributed this to not using an enantiomeric mix of 13-methylpentacosane which they had done for the other alkanes tested, hence they theorised that the stereoisomeric effects might be more influential on the physical properties than first assumed. However it is unknown whether insects synthesise a single enantiomer or a mix of enantiomers, therefore it is unclear as to how important this effect is [19, 28].

In this same study [28] the effect of double bonds in the Z, or *cis*, configuration within the hydrocarbons was investigated and it was found that a double bond reduces the melting point by approximately 50 °C compared to an alkane of the same chain length. Within ant species all double bonds are in the Z, or *cis*, configuration. This introduces an angle into the molecule so the structure is bent, see Figure 1.9. As with the methyl compounds it is thought that this angle disrupts the crystalline packing, and hence lowers the melting point.

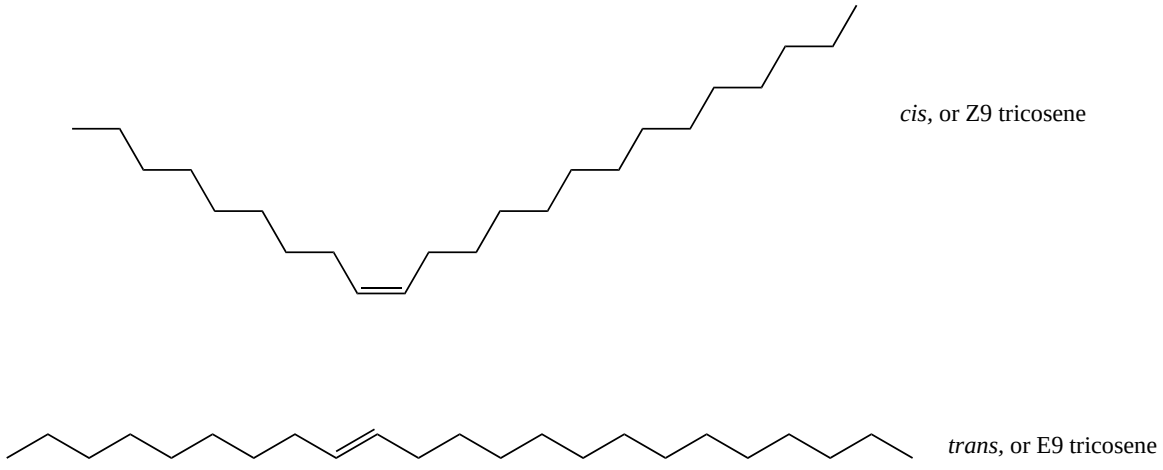


Figure 1.9: The structural differences between isomeric forms of tricosene.

It should be mentioned however that there were two problems with this study; firstly the change in melting point of the alkenes compared to their alkane equivalents was only measured for two alkenes; Z9 tricosene and Z9 heneicosene. Secondly, due to the lack of standards, the effect on the melting point by the position of the double bond was not studied. Instead the authors used melting point data taken from the CRC Handbook for the alkene 1-eicosene and compared it to the published melting point data for eicosane [40]. They found that the published data suggested 1-eicosene melted at 28.5 °C, whereas eicosane melted at 36.8 °C. From this data it was observed that the difference in melting point between an alkene and its equivalent alkane was much greater when the double bond position of the alkene was more central; as in Z9 tricosene, than when it was positioned more terminally (approximately 50 °C, compared to 8 °C). For this reason the authors suggested that the effect of double bond position on melting temperature may be similar to that seen for methyl-branching i.e. the more terminally positioned the double bond, the less the effect. Again the explanation of this is that the effect of the double bond on the packing arrangement is reduced when this bond is positioned more terminally. Likewise when this bond is positioned towards the middle of the molecule the crystalline packing of the molecules is more disrupted.

As previously described the primary purpose of CHCs is to provide the insect with a waterproof layer. Therefore as discussed, if an insect biosynthesises a range of different compound types with multiple chain lengths and properties it will ensure a large solid-liquid phase transition and therefore a flexible waxy layer over a wide range of ambient temperatures [28, 41]. The result is that the chemical profile of ant species tend to show many compounds, although generally between 5 and 50 [27].

1.2.2 Biosynthesis

1.2.2.1 Overview

Whilst the literature shows that there have been many studies on the identification of CHCs [25, 34, 42–44] there have been far fewer on biosynthesis and these tend to focus on species other than ants [45–47]. This is likely due to the size problems associated with working with such small arthropods. Studies of these types frequently involve injecting or feeding the studied species with isotopically labelled compounds, a process which is far easier with larger insect species. These compounds typically feature ^{13}C or ^{14}C labels, the presence of which can be detected within the GC-MS analysed chemical profiles. From studies such as these it has been determined that CHCs are biosynthesised from fatty acids via chain lengthening and subsequent decarboxylation. As Morgan describes in a simplification of the process, the carbon chain of the fatty acid is extended using acetate groups which are first converted to malonate ready for the synthesis [19]. The result of this is a longer chain acyl CoA; this is then decarboxylated to form the desired hydrocarbon. The mechanism is such that the fatty acid is always lengthened to one carbon unit greater than the desired hydrocarbon. This is because the resulting decarboxylation results in the loss of a carbon. This decarboxylation occurs via reduction of the acid to an aldehyde and using cytochrome P_{450}, oxygen is lost as carbon dioxide, as shown in Figure 1.10.

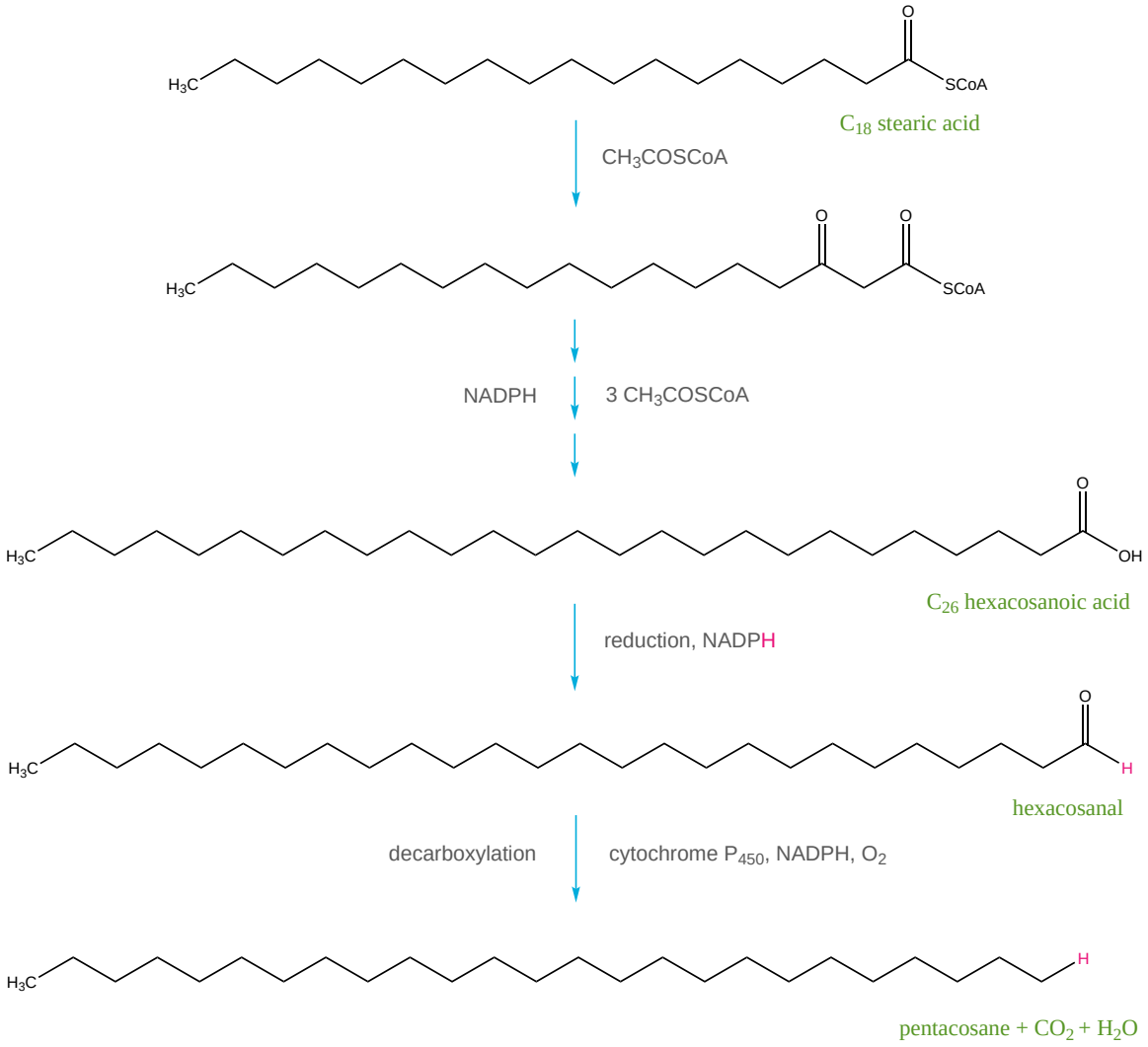


Figure 1.10: Outline biosynthesis of pentacosane via chain lengthening from stearic acid (C18). Adapted from [19].

1.2.2.2 Fatty acids

As discussed in the overview, fatty acids are essential in the biosynthesis of hydrocarbons. These fatty acids can be either obtained through diet, or biosynthesised *de novo*. As a wide range of different fatty acids are required, the biosynthesis of fatty acids is an essential process in the survival of animals. It was in 1907 that JC Collie published research that suggested that fatty acids are produced via the head to tail linking of acetic acid units to form longer chain fatty acids [48]. The formation of the carbon-carbon bonds which link the acetate units together is via a Claisen condensation reaction. This is a relatively straightforward reaction in a chemistry lab; although

requiring a strong base and anhydrous conditions. However in biological organisms this same reaction is possible within an aqueous system at neutral pH. Therefore, in order for this reaction to occur in such conditions, the acetate group must be modified to make it inherently more reactive. This is done by converting the acetate into the thiomalonate derivative. The formation of a malonic thioester greatly increases the acidity of the CH_2 group. Because this group is now more acidic, removal of a proton can occur in relatively neutral conditions and thus the reaction is able to proceed within biological organisms as shown in Figure 1.11, adapted from [19]. The acidity of this CH_2 group can be measured using the acid dissociation constant, or K_a, the greater this number, the greater the extent of dissociation and thus the greater the acidity. As Figure 1.11 shows, the conversion of the acetate into a malonate increases the K_a of the CH_2 group from $3x10^{-25}$ to $1x10^{-13}$. This is because the extra carbonyl group provides additional stabilisation of the resonance molecule. Conversion of the malonate into a thiomalonate increases the K_a value even further; although the precise K_a of the CH_2 group in a thiomalonate is unknown. This is because the sulfur atom, which replaces the oxygen atom, is less electronegative and therefore the formation of the carbanion is more favoured [49]. However it should also be noted that the entire biosynthetic process is also enzyme-mediated and as such is more complex [50]. Within complex organisms biosynthetic processes utilise enzyme complexes known as a megasynthases. Throughout the biosynthetic processes the growing molecules are attached to this complex enzyme, and are only released once the molecule has reached its full length [19]. Throughout this thesis biosynthetic pathways will be represented however for ease the role and attachment of these megasynthases will be simplified.

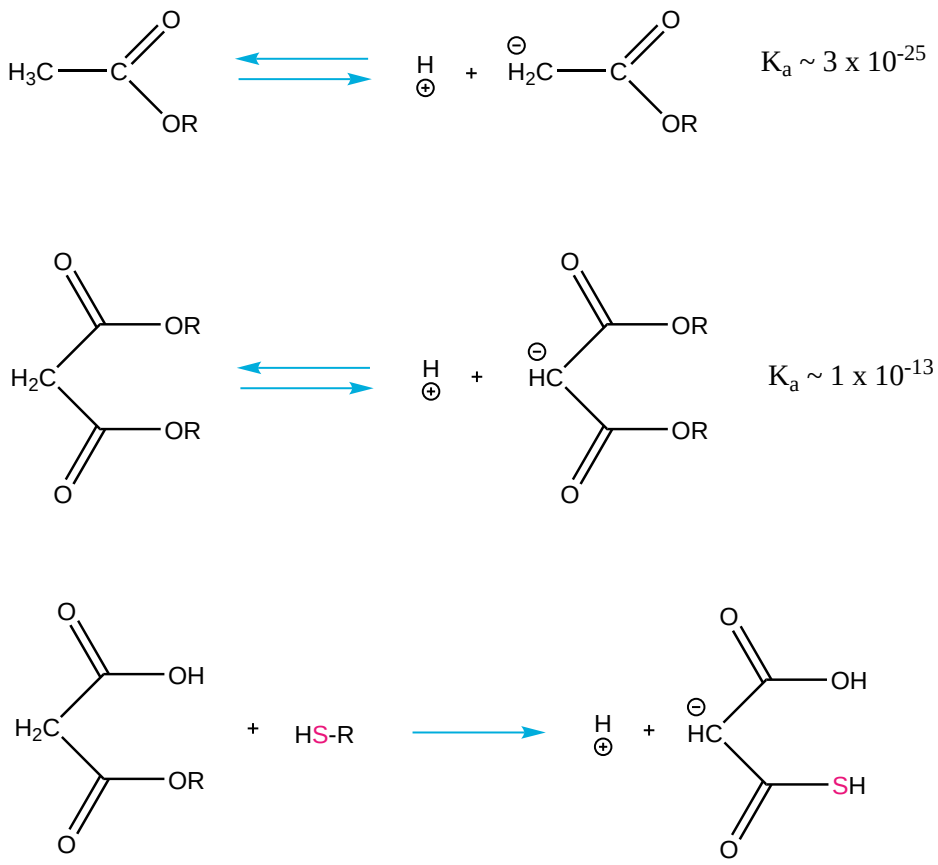


Figure 1.11: Schematic showing how the modification of the acetate unit into a malonate and a thiomalonate (unknown K_a) greatly increases its acidity. Adapted from [19].

The modification of the acetate into a thiomalonate derivative occurs via a carboxylation reaction using the cofactor biotin. This co-enzyme carries the CO_2 which is used to convert acetyl CoA into malonyl CoA. A reaction scheme for this is shown in Figure 1.12 [19, 50, 51]. Note that in this reaction scheme the structure of biotin is simplified. In reality, the part of the molecule indicated in this scheme by biotin, actually consists of the cofactor biotin and the associated biotin carboxyl carrier protein.

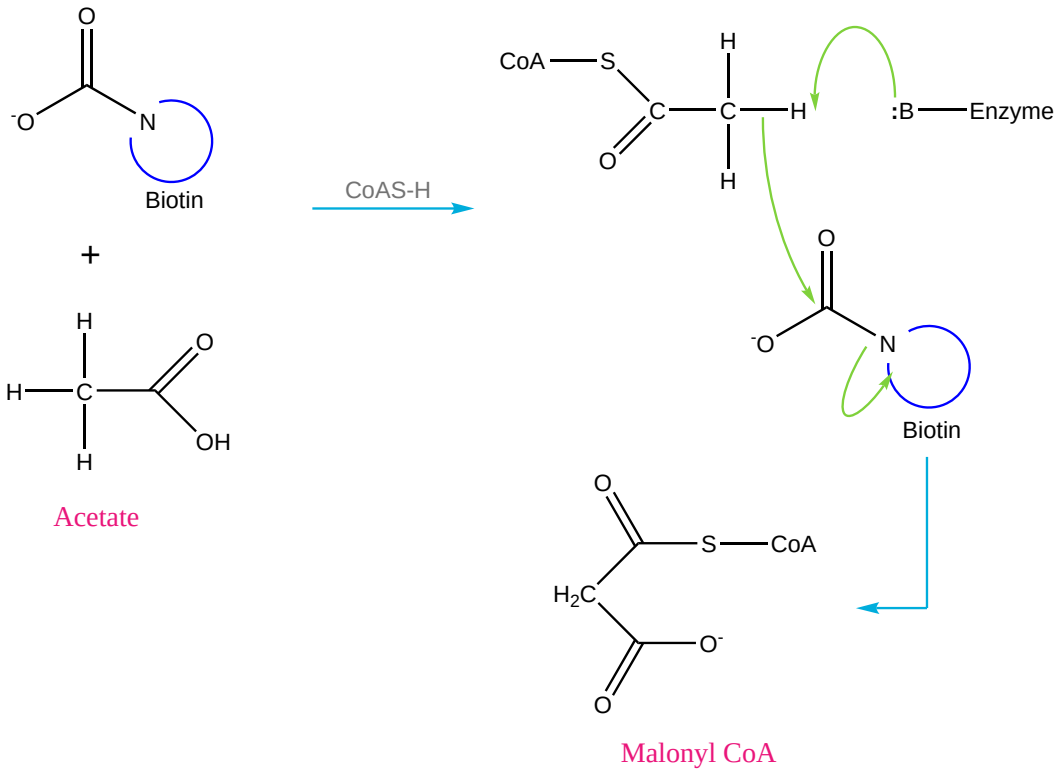


Figure 1.12: Reaction scheme showing the formation of malonyl CoA, note the simplification of the biotin molecule. :B is a basic group on the carboxyl transferase enzyme [19, 50, 51].

The formation of malonyl CoA is a key step in the formation of long chain fatty acids. Once this molecule has been synthesised the reaction can continue. Initially a molecule of malonyl CoA reacts with a molecule of acetyl CoA, to form the new carbon-carbon bond. It is the decarboxylation of the malonate that provides the driving force for the condensation step. These steps, as well as the rest of the mechanism are shown in Figure 1.13. Note that in these mechanisms ACP denotes the acyl carrier protein group which is part of the megasynthase referred to above.

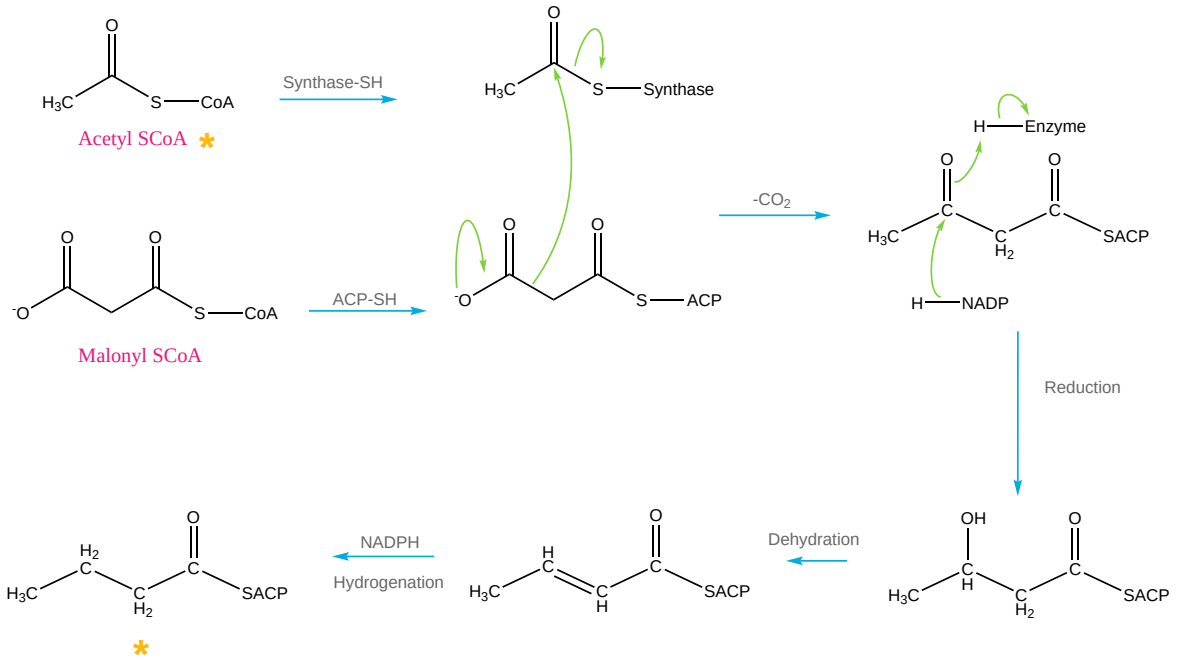


Figure 1.13: Reaction scheme showing the *de novo* formation of a fatty acid using malonyl CoA and acetyl CoA [19, 52]. The product of this reaction indicated by a yellow asterisk can then act as starting material for the next reaction cycle, and thus the chain length is gradually increased.

As shown in Figure 1.13 the reduction, dehydration and hydrogenation steps follow conventional mechanisms and the product of this reaction is labelled with a yellow asterisk. This product then reacts with another molecule of malonyl CoA. This reaction therefore involves several repeating cycles until the desired fatty acid length is reached. Each cycle of the reaction lengthens the chain length by two units, therefore fatty acids with a chain length containing an even number of carbons are by far the most common type.

1.2.2.3 Straight chain hydrocarbons

Straight chain alkanes and alkenes contribute a large proportion of cuticular hydrocarbons found in ant species [27]. The biosynthesis of these compounds is largely the same as that of fatty acids as previously summarised in Figure 1.10. However a more in depth scheme of the chain lengthening steps involved in the biosynthesis of alkanes is demonstrated using the example of nonadecane in Figure 1.14.

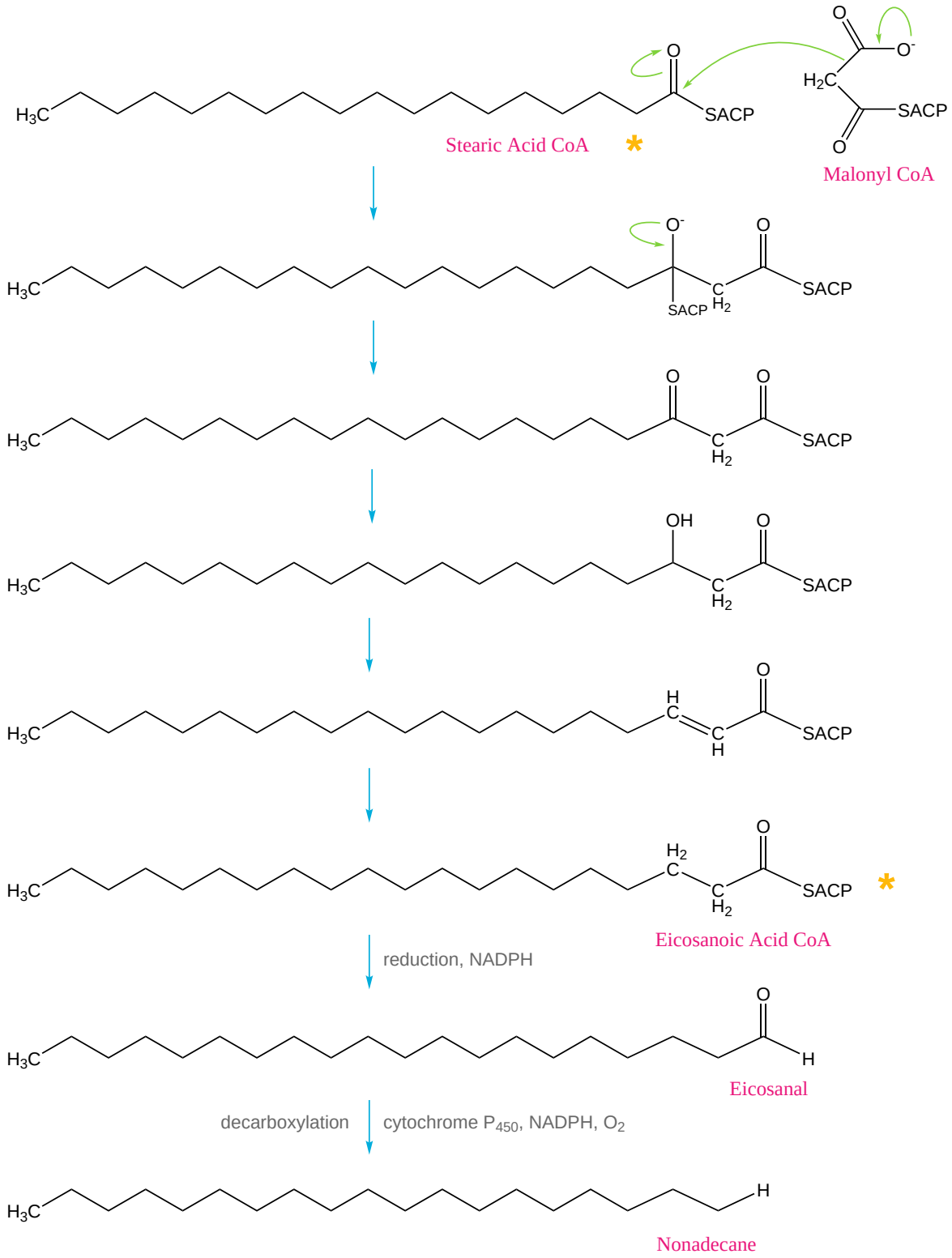


Figure 1.14: Reaction scheme showing the formation of nonadecane using a stearic acid precursor and malonyl CoA. The product of one of the earlier steps indicated by a yellow asterisk can then act as starting material for the next reaction cycle, and thus the chain length is gradually increased. [19, 52].

From this schematic it can be seen that the initial precursor is a suitable fatty acid and as previously seen with the biosynthesis of fatty acids the chain length of the fatty acid is first extended using acetate units (in the form of malonate) until the desired length is exceeded by one carbon unit. This fatty acid then undergoes decarboxylation, to form a straight chain alkane. As before the molecule must undergo several cycles of this extension process to reach the desired length: the yellow asterisk indicates the point at which the product feeds back into the synthesis and can subsequently react with another malonate molecule to increase the chain length. Once the desired chain length is reached, that is one carbon greater than the required alkane length, the product undergoes the end steps to yield the desired alkane.

The biosynthesis of alkenes however is slightly different. In research presented in Howard and Blomquist 2005 [30], the authors suggested that the production of alkenes is via a saturated acyl CoA which is then dehydrogenated using a desaturase enzyme, NADPH, and oxygen. Whilst this may be the case, unpublished work undertaken prior to the start of this research project suggests that the biosynthesis of an unsaturated hydrocarbon can also occur using a suitable unsaturated fatty acid. This therefore shows that insect species can be resourceful in the precursors that they utilise and more research and experimentation is required in this area to determine exactly what compounds can be used as precursors in the biosynthesis of unsaturated CHCs.

1.2.2.4 Methyl-branched hydrocarbons

As well as alkanes and alkenes, another major group of CHCs is methyl-branched hydrocarbons. The formation of this class of compounds is more complex. According to Morgan [19], with the exception of 2-methyl compounds, the methyl-branching is introduced via the exchange of acetic acid for propionic acid (as methylmalonyl CoA). Figure 1.15 shows this process and it can be seen that one molecule of propionate is first converted to methylmalonate before being used during the synthesis. This reacts with

a molecule of acetate, lengthening the carbon chain and adding a methyl branch. Once this methyl-branch is inserted, further lengthening of the carbon chain occurs using only malonate molecules. Additional research has shown through ^{13}Carbon labelling that this methyl group is introduced early in the chain synthesis [19, 52], see Figure 1.15. The extent to which this is true is largely dependant on the required methyl position, for terminally positioned methyl-branches, the methyl part of the chain is formed first. This is then followed by subsequent chain lengthening, in order to form the required compound length.

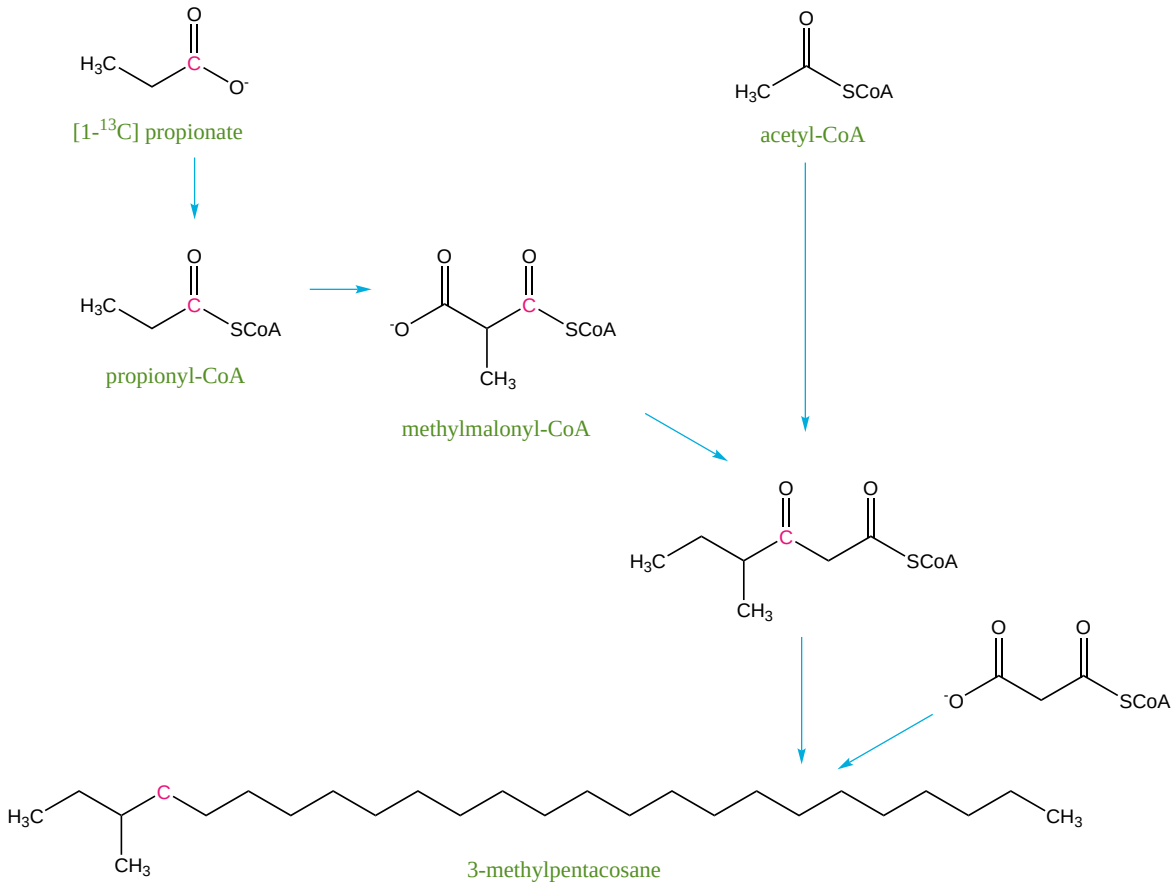


Figure 1.15: Diagram showing the process of incorporation of [1-^{13}C]propionate into 3-methylpentacosane. Adapted from [52].

In this instance the introduction of the methyl-branch is achieved by lengthening the chain with a unit of methylmalonate at the correct point during the chain elongation [19, 52]. The precursor for methylmalonate is propionate; in the same way the

precursor for malonate is acetate. However methylmalonate can also be biosynthesised from succinate in insect species with very high levels of vitamin B_{12}, for example termites, which have naturally high levels of this vitamin. They are therefore capable of producing methylmalonate via a free radical arrangement initiated by a co-enzyme form of B_{12}. The majority of insect species however lack the necessary levels of vitamin B_{12} for this reaction. Instead these species can also use the amino acids valine, isoleucine and methionine as precursors for the formation of methylmalonate [19].

A small number of ant species biosynthesise 2-methyl alkanes [27]. The biosynthesis of these compounds occurs via a slightly different route, instead of fatty acid elongation the precursors for 2-methyl hydrocarbons are either valine or leucine. The amino acid which is used depends on whether an odd chain hydrocarbon or an even chain hydrocarbon is to be synthesised. The use of valine leads to an even number of carbons in the chain, whilst the use of leucine leads to an odd number.

It also seems that, as Tillman et al. [53] suggest, insects are able to use a variety of suitable compounds to act as precursors, depending on their dietary intake. Whilst propionate can be used as the source of methyl-branching, it can also be metabolised to form acetate units as Dillwith et al. discovered when they found the labelled ^{13}C of [2-^{13}C] and [3-^{13}C]propionate in the ^{13}C NMR spectra of straight chain alkenes from the housefly *Musca domestica* [54]. In similar fashion a variety of amino acids can be metabolised to form propionyl-CoA. In the above study the authors also fed [3,4,5-$^{13}C_3$]valine to female houseflies and found that the labelled hydrocarbons were incorporated into the methyl-branched hydrocarbons [54]. As with all studies of this type the main limitation is the labelled precursors; these can often be prohibitively expensive or difficult to obtain or synthesise. This means that the choice of labelled precursors available for use is limited, and therefore experiments often have to be constrained due to budgetary or practical limitations.

1.3 Rationale of Research

Ants can be classed as a truly eusocial species. Underpinning this characteristic is a strong system of kin recognition whereby individuals can easily distinguish between nestmates and non-nestmates in order to appropriately direct altruistic behaviours. The basis of this recognition system is a chemical profile present on the ants' cuticle which consists of a blend of long chain hydrocarbons. The exact compounds present within this profile varies between species, whilst the ratios of the compounds varies between colonies of the same species. As such, continued specific production of these compounds is essential; a process which relies on sophisticated biosynthetic routes within the ants and the biotransformation of simple molecular units into more complex longer chain hydrocarbons. The biosynthesis of these molecules has been previously researched in a variety of species, including cockroaches [45–47]. However this type of research is yet to be performed on ant species, and generally the biosynthesis of cuticular hydrocarbons in ants is still relatively poorly understood. This may be due to the inherent issues surrounding working with ants. They are physically small, and therefore harder to work with. They are also much more genetically diverse, compared to, for example, cockroaches, which can be bred specially for lab purposes and hence have known genetics.

One of the simplest ways of analysing biosynthetic routes is through the use of labelled substrate precursors. These precursors are chemically labelled with one or more deuterium, or more commonly, ^{13}C atoms. The incorporation of such a label results in the precursor having a slightly greater mass than the unlabelled equivalent. If the ant uses this precursor to biosynthesise a hydrocarbon, then this extra mass can be identified in the final hydrocarbon product. Thus it is possible to offer a variety of different precursor compounds, with labels in a variety of different positions and see which are identified in the final hydrocarbon. This will provide some insight into the biosynthesis of key compounds for various species of British ant. The findings from

these studies can then be compared against the literature to see if the models based on other species are correct. The precursors used within this work include simple acetate and propionate molecules, amino acids, and more complex unsaturated fatty acids.

The main aim of this project therefore was to use feeding experiments and labelled substrates to aid determination of the possible biosynthetic routes in a variety of British ant species. By adding compounds to the ants' usual diet which have isotopically labelled carbons or hydrogens, the final incorporation of this molecule into the cuticular chemistry was studied. It was hoped this would allow us to understand how the ants biosynthesise CHCs and what precursors they can use. The ultimate aim of this project was to elucidate the biosynthetic routes employed by our ant species by testing the already established biosynthetic models obtained from other insect species, to see if these hold true for a variety of British ant species.

References

[1] Gullan PJ, and Cranston PS, *The Insects: An Outline of Entomology*, (Chapman and Hall, 1994)

[2] Sudd JH, and Franks NR, *The Behavioural Ecology of Ants*, (Blackie, 1987)

[3] Crespi BJ, and Yanega D, 'The definition of eusocialty,' (1995), *Behav. Ecol.*, 6(1), 109-115

[4] Bolton B, Antweb at http://www.antweb.org/description.do?rank=family&name=formicidae&project=allantwebants (accessed January 2014)

[5] Schultz TR, 'In search of ant ancestors,' (2000), *PNAS*, 97(26), 14028-14029

[6] Bees, Wasps & Ants Recording Society 2016 at http://www.bwars.com/taxonomy/term/9 (accessed June 2018)

[7] Skinner G, and Allen GW, *Ants*, (The Richmond Publishing Company Ltd, 1996)

[8] Wilson EO, and Fagen RM, 'On the estimation of total behavioral repertories in Ants,' *J. N. Y. Entomol. Soc.*, (1974), 82, 106-112

[9] Hamilton WD, 'The genetical evolution of social behaviour I,' (1964), *J. Theoret. Biol.*, 7, 1-16

[10] Hamilton WD, 'The genetical evolution of social behaviour II,' (1964), *J. Theoret. Biol.*, 7, 17-52

[11] Zinck L, Châline N, and Jaisson P, 'Absence of nepotism in worker-queen care in polygynous colonies of the ant *Ectatomma tuberculatum*,' (2009), *J. Insect. Behav.*, 2(3), 196-204

[12] Hannonen M, and Sundström L, 'Worker nepotism among polygynous ants,' (2003), *Nature*, 421, 910

[13] Keller L, 'Indiscriminate altruism: unduly nice parents and siblings,' (1997), *Trends Ecol. Evol.*, 12, 99-103

[14] DeHeer CJ, and Roos KG, 'Lack of detectable nepotism in multiple-queen colonies of the fire ant *Solenopsis invicta* (Hymenoptera: Formicidae),' (1997), *Behav. Ecol. Sociobiol,*, 40, 27-33

[15] Ratnieks FLW, and Reeve HK, 'Conflict in single-queen hymenopteran societies: the structure of conflict and processes that reduce conflict in advanced eusocial species,' (1992), *J. Theor. Biol.*, 158, 33-65

[16] Sherman PW, Reeve HK, and Pfennig DW, *Recognition systems*, In 'Behavioural Ecology, An Evolutionary Approach' (Krebs JR and Davies NB) (Blackwell Science, 1997)

[17] Reeve HK, *Game theory, reproductive skew and nepotism*, In 'Game theory and animal behaviour' (Dugatkin L and Reeve HK) (Oxford University Press, 1998)

[18] Van Zweden JS, Brask JB, Christensen JH, Boomsma JJ, Linksvayer TA, and D'Ettore P, 'Blending of heritable recognition cues among ant nestmates creates distinct colony gestalt odours but prevents within-colony nepotism,' (2010), *J. Evol. Biol.*, 23, 1498-1508

[19] Morgan ED, *Biosynthesis in Insects*, (Royal Society of Chemistry, 2010)

[20] Soroker V, Vienne C, and Hefetz A, 'Hydrocarbon dynamics within and between nestmates in *Cataglyphis niger* (Hymenoptera: Formicidae),' (1995), *J. Chem. Ecol.*, 21(3), 365-378

[21] Crozier RH, and Dix MW, 'Analysis of two genetic models for the innate components of colony odor in social Hymenoptera,' (1979), *Behav. Ecol. Sociobiol.*, 4(3), 217-224

[22] Martin SJ, and Drijfhout FP, 'Nestmate and task cues are influenced and encoded differently within ant cuticular hydrocarbon profiles,' (2009), *J. Chem. Ecol.*, 35(3), 368-74

[23] Lockey KH, 'Lipids of the insect cuticle: Origin, composition and function,' (1988), *Comp. Biochem. Physiol.*, 89B(4), 595-645

[24] Greene MJ, and Gordon DM, 'Structural complexity of chemical recognition cues affects the perception of group membership in the ants *Linephithema humile* and *Aphaenogaster cockerelli*,' (2007), *J. Exp. Biol.*, 210(5), 897-905

[25] Akino T, 'Cuticular Hydrocarbons of *Formica truncorum* (Hymenoptera: Formicidae): Description of new very long chained hydrocarbon components,' (2006), *Appl. Entomol. Zool.*, 41(4), 667-677

[26] Kaal E, and Janssen HG, 'Extending the molecular application range of gas chromatography,' (2008), *J. Chromatogr. A.*, 1184(1-2), 43-60

[27] Martin S, and Drijfhout F, 'A review of ant cuticular hydrocarbons,' (2009), *J. Chem. Ecol.*, 35(10), 1151-1161

[28] Gibbs A, and Pomonist JG, 'Physical properties of insect cuticular hydrocarbons: The effects of chain length, methyl-branching and unsaturation,' (1995), *Comp. Biochem Physiol.*, 112B(2), 243-249

[29] Blomquist GJ, Nelson DR, and De Renobales M, 'Chemistry, biochemistry and physiology of insect cuticular lipids,' (1987) *Arch. Insect Biochem. Physiol.*, 6(4), 227-265

[30] Howard RW, and Blomquist GJ, 'Ecological, behavioural, and biochemical aspects of insect hydrocarbons,' (2005), *Annu. Rev. Entomol.*, 50, 371-393

[31] Greene MJ, and Gordon DM, 'Cuticular hydrocarbons inform task decisions,' (2003), *Nature*, 423, 32

[32] Martin SJ, Vitikainen E, Helanterä H, and Drijfhout FP, Chemical basis of nestmate discrimination in the ant *Formica exsecta*,' (2008), *Proc. R. Soc. B*, 275, 1271-1278

[33] Guillem RM, Drijfhout FP, and Martin SJ, 'Using chemo-taxonomy of host ants to help conserve the large blue butterfly,' (2012), *Biol. Conserv.*, 148(1), 39-43

[34] Urech R, Brown GW, Moore CJ, and Green PE, 'Cuticular hydrocarbons of the buffalo fly, *Haematobia exigua* and chemotaxonomic differentiation from horn fly *H. irritans*,' (2005), *J. Chem. Ecol.*, 31(10), 2451-2461

[35] Martin SJ, Helanterä H, and Drijfhout FP, 'Evolution of species-specific cuticular hydrocarbon patterns in *Formica* ants,' (2008), *Biol. J. Linn. Soc.*, 95(1), 131-140

[36] Nelson DR, Tissot M, Nelson LJ, Fatland CL, and Gordon DM, 'Novel wax esters and hydrocarbons in the cuticular surface lipids of the red harvester ant, *Pogonomyrmex barbatus*,' (2001), *Comp. Biochem. Physiol.*, 128B(3), 575-595

[37] Martin SJ, Zhong W, and Drijfhout FP, 'Long-term stability of hornet cuticular hydrocarbons facilitates chemotaxonomy using museum specimens,' (2009), *Biol. J. Linn. Soc.*, 96, 732-737

[38] Moore HE, Adam CD, and Drijfhout FP, 'Potential use of hydrocarbons for aging *Lucilia sericata* blowfly larvae to establish the postmortem interval,' (2012), *J. Forensic Sci.*, 58, 1-9

[39] Gibbs AG, 'Water-proofing properties of cuticular lipids,' (1998), *Integr. Comp. Biol.*, 38, 471-482

[40] Lide DR, *CRC Handbook of Chemistry and Physics 73rd ed.*, (CRC Press, 1992)

[41] Drijfhout FP, Kather R, and Martin SJ, *The role of cuticular hydrocarbons in insects*, In 'Behavioural and Chemical Ecology' (Zhang W and Liu H) (Nova Science Publishers Inc., 2010).

[42] Howard RW, Pérez-Lachaud G, and Lachaud J-P, 'Cuticular hydrocarbons of *Kapala sulcifacies* (Hymenoptera: Eucharitidae) and its host, the Ponerine ant *Ectatomma ruidam* (Hymenoptera: Formicidae),' (1994), *Ann. Entomol. Soc. Am.*, 94(5), 707-716

[43] Brown WV, Rose HA, Lacey MJ, and Wright K, 'The cuticular hydrocarbons of the giant soil-burrowing cockroach *Macropanesthia rhinoceros* Saussure (Blattodea: Blaberidae: Geoscapheinae): analysis with respect to age, sex and location,' (2000), *Comp. Biochem. Physiol. B. Biochem. Mol. Biol.*, 127(3), 261-277

[44] Bagnères AG, and Morgan ED, 'A simple method for analysis of insect cuticular hydrocarbons,' (1990), *J. Chem. Ecol.*, 16(12), 3263-3276

[45] Gu X, Quilici D, Juarez P, Blomquist GJ, and Schal C, 'Biosynthesis of hydrocarbons and contact sex pheromone and their transport by Lipophorin in females of the German cockroach *Blattella germanica*,' (1995), *J. Insect Physiol.*, 41(3), 257-267

[46] Chase J, Jurenka RA, Schal C, Halarnka PP, and Blomquist GJ, 'Biosynthesis of methyl-branched hydrocarbons of the German cockroach *Blattella germanica* (L.) (Orthoptera Blattellidae),' (1990), *Insect Biochem.*, 20(2), 149-156

[47] Young HP, and Schal C, 'Cuticular hydrocarbon synthesis in relation to feeding and developmental stage in nymphs of *Blattella germanica* (Dictyoptera: Blattellidae),' (1997), *Ann. Entomol. Soc. Am.*, 90(5), 655-663

[48] Collie JN, 'Derivatives of the multiple keten group,' (1907) *J. Chem. Soc.*, 91, 1806-1813

[49] Hunt I, *Chapter 21: Ester Enolates*, University of Calgary, at http://www.chem.ucalgary.ca/courses/350/Carey5th/Ch21/ch21-2.html

[50] Mann J, *Chemical Aspects of Biosynthesis*, (Oxford University Press, 1994)

References

[51] Campbell MK, and Farrell SO, *Biochemistry: 8th Edition*, (Cengage Learning, 2015)

[52] Blomquist GJ, *Insect Hydrocarbons*, (Cambridge University Press, 2010)

[53] Tillman JA, Seybold SJ, Jurenka RA, and Blomquist GJ, 'Insect pheromones–an overview of biosynthesis and endocrine regulation,' (1999), *Insect Biochem. Mol. Biol.*, 29(6), 481-514

[54] Dillwith JW, Nelson JH, Pomonis JG, Nelson DR, and Blomquist GJ, 'A ^{13}C NMR study of methyl-branched hydrocarbon biosynthesis in the housefly,' (1982), *J. Biol. Chem.*, 257(19), 11305-11314

Chapter 2

Chemical Techniques & Analysis

2.1 Overview

The basis of the work presented in this thesis is underpinned by the extraction of the cuticular hydrocarbons secreted by ants. This process is followed by chemical analysis and then statistical analysis of the results to determine any differences between sample sets. The flowchart found in Figure 2.1 summarises the overall process, and further information about each step can be found in the relevant sections.

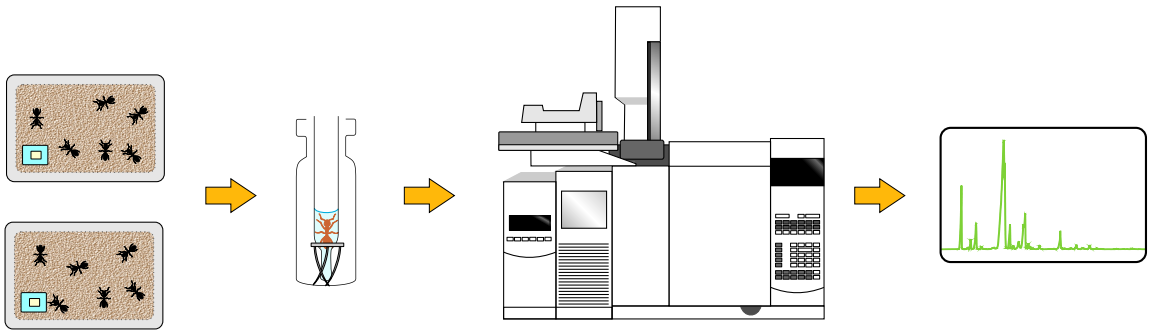


Figure 2.1: A schematic showing an overview of the experimental process underpinning this project. The process starts with several experimental sub-populations of ants. The CHCs are extracted and chemically analysed using a gas chromatograph, which produces a chromatogram. Following this the results are analysed using statistical methods to reveal any subtle differences (not shown).

2.2 Extraction Techniques

2.2.1 Whole body extraction

In order to study the cuticular hydrocarbon (CHC) profiles of insects there must first be a robust method to remove the non-polar cuticular hydrocarbons. This is known as the extraction method and it is important to consider the technique used for this step. Traditionally whole insects are immersed in a non-polar chemical solvent such as *n*-pentane or *n*-hexane [1] within a small glass vial. This effectively dissolves the non-polar CHCs into the solvent, which is then evaporated to dryness. The final step within the process is to re-add the minimum amount of solvent, typically 30 μL, immediately prior to chemical analysis. This technique is widely used by many researchers for the analysis of cuticular hydrocarbons in both ant and non-ant species [2–6], however it is difficult to quantifiably evaluate the extraction method [1]. There is also the issue of inadvertently extracting internal lipids from within the organism, thought to be caused by submerging the insect within the solvent for too long. A review of the literature suggests that different research groups have their own tried and trusted extraction durations. For example Hay-Roe et al. extracted the cuticular hydrocarbons from whole butterflies by rinsing the insects with *n*-pentane [7], whilst Mullen et al. extracted CHCs from whole crickets by immersing them in *n*-hexane for 5 minutes [8]. Finally Martin et al. extracted hornet eggs and wings by immersing them for 30 minutes in *n*-hexane [9].

A comprehensive study by Vander Meer et al. [10] investigated the effects of different extraction times on the resulting CHC profiles of the ant *Solenopsis invicta*. In this study the author pooled 100 of the insects together and extracted them in *n*-hexane for the following time periods; 3, 5, 7, 10, and 15 minutes, and overnight. At each time point aliquots of hexane were removed, isolated to remove contaminants and analysed. In order to evaluate the effect that this had on the results, firstly the profile of the ant species was considered and the five most abundant peaks selected for analysis. At

each time interval the percentage composition of the profile was determined in terms of just these five chosen peaks. In addition to this the mass of the insects was measured before and after extraction and the mass differences compared. This was in order to identify how much effect extraction time had on the amount of cuticular hydrocarbons extracted.

The results of the study found that the percentage abundances of the five selected peaks were similar for the time periods 5, 7, 10, and 15 minutes. For the shortest and longest extraction times however there was a bigger change. It was found that a short extraction time (3 minutes) was less effective at removing the two dimethyl compounds; the compounds with the greatest mass. Consequently these accounted for less of the resulting profile. It was also found that the compound with the lowest mass, *n*-heptacosane, contributed to much higher percentage of the profile. For the overnight extraction *n*-heptacosane accounted for far less of the profile whilst there was far more 13,15-dimethylheptacosane extracted. This research therefore indicated that longer time periods were more effective at removing the heavier hydrocarbons.

Analysis of the masses prior to and after extraction showed a range of mass differences from 31.8 to 46.4 μg for the extraction lengths up to 15 minutes, with the amount of hydrocarbons extracted increasing with the extraction time. However for overnight extractions the mass difference was much greater, at 138.8 μg. This indicated that overnight extraction was removing far more mass than when the insects were immersed for a period of up to 15 minutes. The authors suggested that this was due to the extraction of internal compounds, most likely from the postpharyngeal gland, and that to avoid this the insect should not be left submerged overnight. However it is not clear if, when these samples were left overnight, the solvent itself completely evaporated; exposure to air would have caused the insect to rapidly desiccate leading to exaggerated mass loss.

The study also did not look at the effect of leaving the insects immersed for a period of 1-2 hours. This is typically the length of time that it takes for an insert of hexane (approximately 200 μL) to evaporate, this would have been an interesting measurement to have made as it would negate the need to remove the insect from the vial and therefore prevent loss of some of the extracted hydrocarbons. Based on these results the authors suggested that 7 minutes was the optimum extraction time, however this means the addition of an extra step i.e. the removal of the insects [10].

Due to its efficiency and the reasons described above the whole body extraction method was the chosen technique for the duration of this project. However there are other alternative processes that can be used to extract cuticular hydrocarbons. These will be explained in further detail.

2.2.2 Solid sample injection

Whole body or partial extractions are not the only method that can be used to remove the hydrocarbons from an insect's cuticle. One novel extraction technique that does not require immersion in a solvent is that first developed by Morgan in 1972 [11]. This extraction method utilises a device known as a solid sample injector; totally removing the need for *n*-pentane or *n*-hexane. In this technique the samples, normally whole or partial insects, are sealed within a glass capillary which is placed within the solid sample injector. This is then lowered into the injection port of the gas chromatograph (used for the chemical analysis) and heated for several minutes. The sealed glass capillary is then crushed, at which point the already vaporised cuticular hydrocarbons and other volatile compounds are released into the carrier gas stream within the instrument [11]. The benefits of using this device are numerous; as there is no solvent involved there is no large initial peak attributed to the solvent, which can mask the signal of rapidly eluting volatile compounds. Secondly as the extracted compounds are not dissolved in a solvent they are not diluted, therefore this technique is particularly

suited to the analysis of trace compounds. Finally it is useful for studies focussing on internal compounds; these are often very volatile and can be easily lost from standard liquid extractions. For a diagram of a solid sampler device see Figure 2.2.

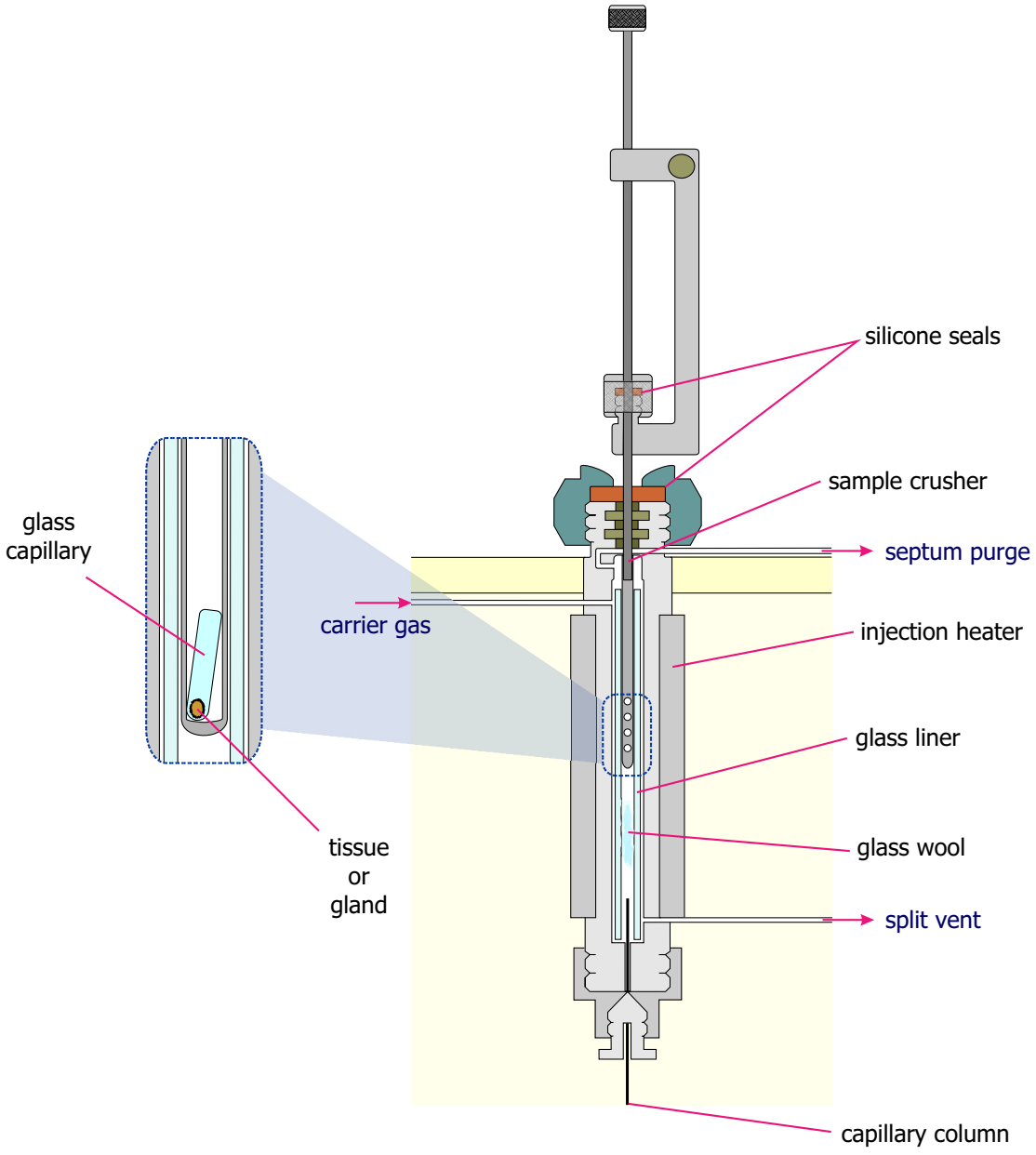


Figure 2.2: Diagram of a solid sampler device adapted from [11]. It can be seen that when the sample, sealed in a glass capillary, is crushed, the vaporised sample can enter the gas flow.

.

The capability of this technique was further investigated by Bagnères and Morgan in 1990 [12]. In this study the authors used this technique to determine the chemical profile of specific areas of individual insects by dissecting small pieces of cuticle from various insect species and sealing them within a glass capillary. The study found that the best results were obtained when the sample was heated to 250 °C for 4 minutes before crushing the capillary. Using this technique the authors were able to obtain a gas chromatogram from a single leg of the ant species *Manica rubida* which had just 0.84 μg of hydrocarbons present on the cuticle. This result also showed that the chemical profile obtained from this sample, using the solid sampler device, was comparable to that obtained via a whole body extraction in a previous study [13]. Although not analysed, the authors felt that this technique was sensitive enough to be used even with single segments of leg.

However there are several drawbacks to this method, in particular the insect must be carefully prepared and sealed in a glass capillary, a process that can be time consuming. Additionally the technique requires the use of a solid sampler injector, and as this technique involves crushing a glass capillary, it can lead to debris in and around the injection port. Finally there is no means of using an automated sampler with this technique, therefore a large series of samples must be individually prepared and injected manually, a process which is time consuming.

2.2.3 Solid phase micro extraction

The final method that can be used is Solid Phase Micro Extraction, known as SPME. This technique involves the chemical adsorption of volatile compounds onto a retractable fibre. This fibre is then inserted directly into the injection port of a gas chromatograph (GC) and the compounds thermally desorbed. The fibre can be coated with a variety of different adsorbent materials depending on the compounds to be extracted. Damage to the retractable fibre is prevented by housing it within a needle,

this needle pierces the septum of the injection port and the fibre is then extended. By exposing the fibre to the high temperatures of the injection port the adsorbed compounds are volatilised and carried into the carrier gas flow. The fibre is held within the port for a short period of time, usually a few minutes. As this process totally removes the cuticular hydrocarbons, each fibre can be reused several times [14, 15], see Figure 2.3.

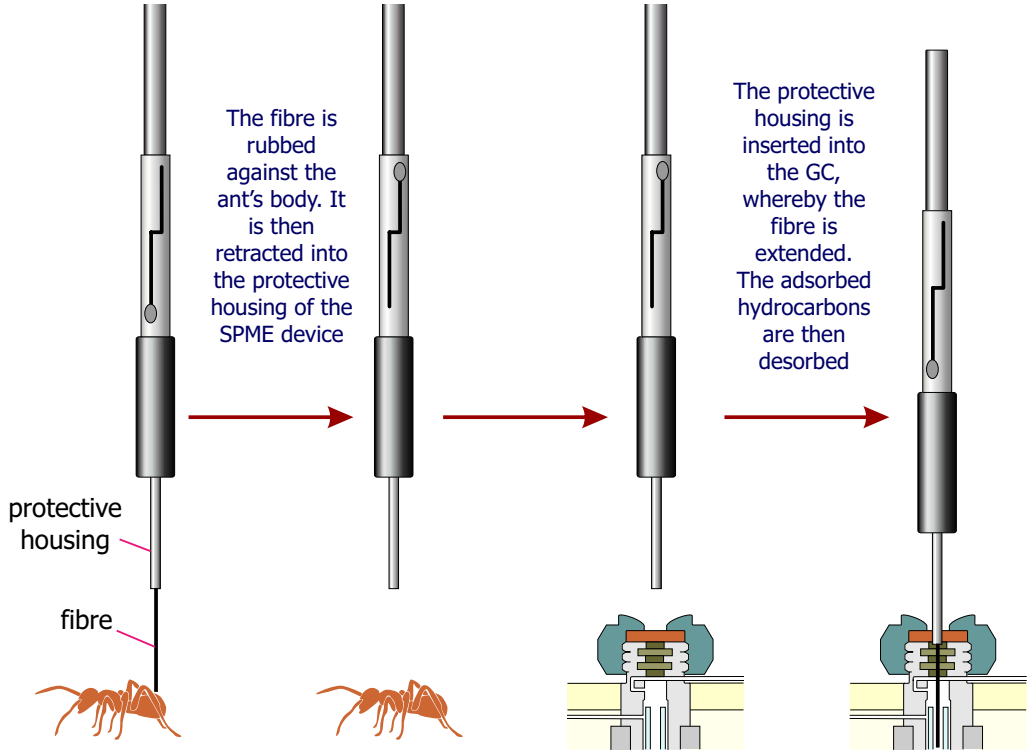


Figure 2.3: Schematic of the SPME process, showing how the cuticular hydrocarbons are extracted from the insect's body and analysed using GC.

Within insect studies, SPME can be used in two different ways. Either the fibre can be directly rubbed against the insect to facilitate the adsorption of the hydrocarbons, or it can be used to sample more volatile compounds such as pheromones, using a headspace based technique [15]. One study utilising the former technique is that by Monnin et al. [16], where the authors used SPME to sample live *Dinoponera* workers. This was done by rubbing the polydimethylsiloxane fibre (suitable for high molecular weight hydrocarbons) against the ant in between the 6th and 7th abdominal segment

for 2 minutes, before introducing the fibre to the GC-FID port at 280 °C. The benefits of this are that the same individual can be sampled multiple times, as unlike liquid extraction this is not a lethal technique. Although these fibres can be reused several times care should be exercised when using SPME as the fibres themselves are very brittle and have a tendency to snap if not carefully handled, as the authors themselves found [16].

The results of this study indicated that SPME, when compared to a liquid extraction using *n*-pentane, tended to over-extract the lower molecular weight compounds. Specifically the results of the study indicated that the amount of *n*-C23 in the resulting chromatogram accounted for 43% of the chemical profile via a SPME extraction and just 26% for an extraction using *n*-pentane. Conversely a SPME extraction under-sampled the heavier compounds, (the amounts of *n*-C32 totalling 13% vs 24% for extractions with SPME and *n*-pentane respectively) [16]. However the authors did not investigate this tendency further. This bias could have been caused by two variables. Perhaps the fibre itself was preferentially adsorbing the lower molecular weight compounds. This could have been tested by changing the fibre type or the length of sampling time. Additionally this result could have been caused by the injection port. The port was only heated to 280 °C; investigation of higher injection port temperatures may have caused better volatilisation of the hydrocarbons from the fibre and hence results closer to those obtained via whole body extractions. However as previously discussed SPME sampling tends to give cleaner hydrocarbon profiles as there is no chance that internal compounds are extracted. The decision to use SPME is therefore generally based on whether repeated sampling is required as this is by far the biggest benefit to using this technique.

For headspace SPME the methodology is slightly different in that the fibre is inserted through a septum into the headspace of a vessel containing the organism to be studied. The fibre is held there for a period of time (minutes to hours) before being retracted

and, in the same way as before, injected into the GC [15]. This technique has more limited applications, however it has been used within research to study both chemicals produced by plants and in flavour analysis [17, 18]. As the remit of this study is generally not concerned with volatile compounds this type of SPME is less applicable.

2.3 Instrumentation

2.3.1 Overview

Following the chemical removal of the cuticular hydrocarbons the extract must be analysed. There are several steps involved in this process. The chemical profile of CHCs that is removed from the ant is complex and contains many different compounds. The first step in this process therefore is the separation of the compounds within the extract. Following this separation identification can take place. Each of these steps and the instrumentation and techniques that can be used for each will be discussed in the following sections.

2.3.2 Chromatography

Chromatography refers to a process used to separate compounds within a mixture. The term chromatography (literally colour writing) was first used by Michael Tswett in the early twentieth century. Often referred to as the "father of chromatography," Tswett was able to separate out the pigments found within plant leaves [14]. Although the term chromatography was first applied with reference to the separation of coloured compounds, there now exist many types of chromatography, with a variety of separation mechanisms. Despite this they all rely on exploiting differences in the physical characteristics of the compounds to be separated. These physical characteristics can include, but are not limited to, molecular size or shape, charge, volatility and adsorption properties [19]. Whilst there are many different types of chromatography, they all rely on the same essential components. Firstly there must be a stationary phase present, this can be a solid, liquid or gel which is supported and held in place by a matrix. There must also be a mobile phase present. This can be a liquid or a gas and it acts as a vehicle to carry the sample mixture through the stationary phase in order for separation to be achieved. The underlying principle of chromatography is that as

the mobile phase carries the sample through the stationary phase the components are separated. The exact mechanism of the separation can vary however [19].

2.3.3 Gas Chromatography

2.3.3.1 Overview

Gas Chromatography (GC) is one of the most widely used analytical techniques in chemical sciences [22]. The instrument comprises four main components; the injector, the column, the oven and the detector. A basic schematic of a GC instrument is shown in Figure 2.4.

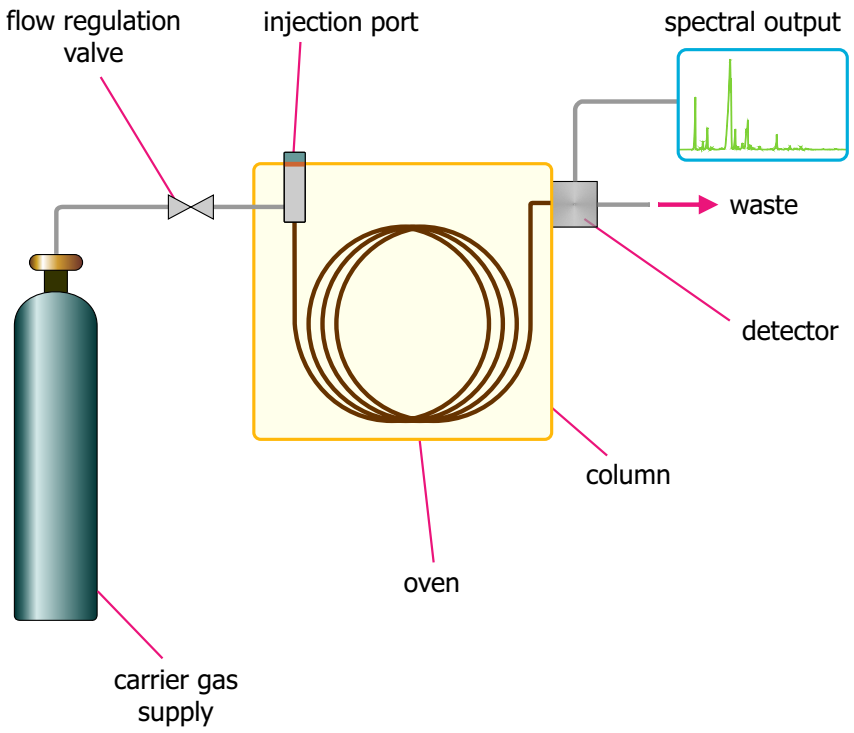


Figure 2.4: Schematic showing the basic components of a GC instrument.

GC analysis begins when a few microlitres of sample is injected using a syringe and rapidly volatilised in the injection port where it then mixes with an inert gas, known as the carrier gas. This gas also acts as the mobile phase and carries the volatilised sample through a fine capillary column, which is typically 15-30 m in length [14]. The

column is coiled inside the GC oven, which allows for variable, accurate temperature control. The flow of the carrier gas can be precisely controlled in order to regulate the speed which the analytes pass through the column. As the individual components travel along the column they are separated from each other based on their relative boiling points as well as their affinities with the stationary phase, via a mechanism known as partition chromatography, see Figure 2.5. As Figure 2.5 shows, the greater the affinity of a particular compound to the mobile phase the quicker it will travel through the column and the earlier it will reach the detector. Compounds with less affinity for the mobile phase will travel much slower through the column and reach the detector later. In GC the stationary phase is a thin film of liquid coated onto the inside wall of a capillary column. Once separated out the analytes pass through a detector, of which there are several types, before purging into the atmosphere. It is important to note that GC separation is only suitable for samples which contain volatile compounds, typically those with boiling points below 500 °C [14, 23]. Non-volatile compounds require derivatisation prior to injection.

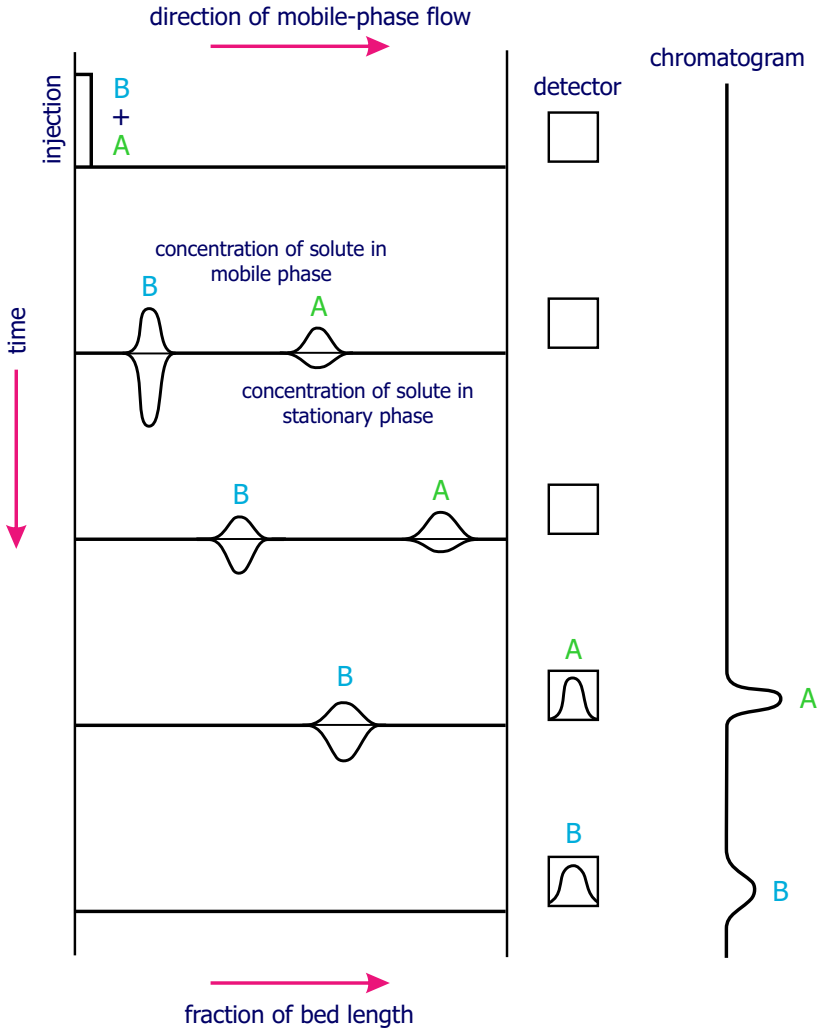


Figure 2.5: Schematic of the chromatographic process adapted from [23].

2.3.3.2 Injection

The primary function of the injection port is to vaporise and introduce the gaseous sample to the column. There are several different types of port, the choice of which depends on the sample and the requirements. The temperature of the port is set so as to volatilise the sample effectively, however it should be noted that increasing the temperature of the injection port can lead to the thermal degradation of some compounds [14]. With the analysis of hydrocarbons this is generally less of an issue as they are thermally stable and do not undergo chemical change upon heating.

Most modern GC systems utilise a split/splitless injector, which is an injector that can be operated in either split or splitless mode. See Figure 2.6 for a diagram of a typical split/splitless injector. In split mode the carrier gas arrives in the vaporisation chamber with a relatively large flow, whereby it mixes with the vaporised sample. Within this chamber a split valve then separates and diverts the larger portion of the carrier gas and sample to the waste outlet. The smaller portion enters the column, the exact amount depending on the split ratio, or the dilution factor required with that sample. Using the split ratio, concentrated samples can be introduced into the column without the analyst having to worry about damaging the column through overloading [22, 24].

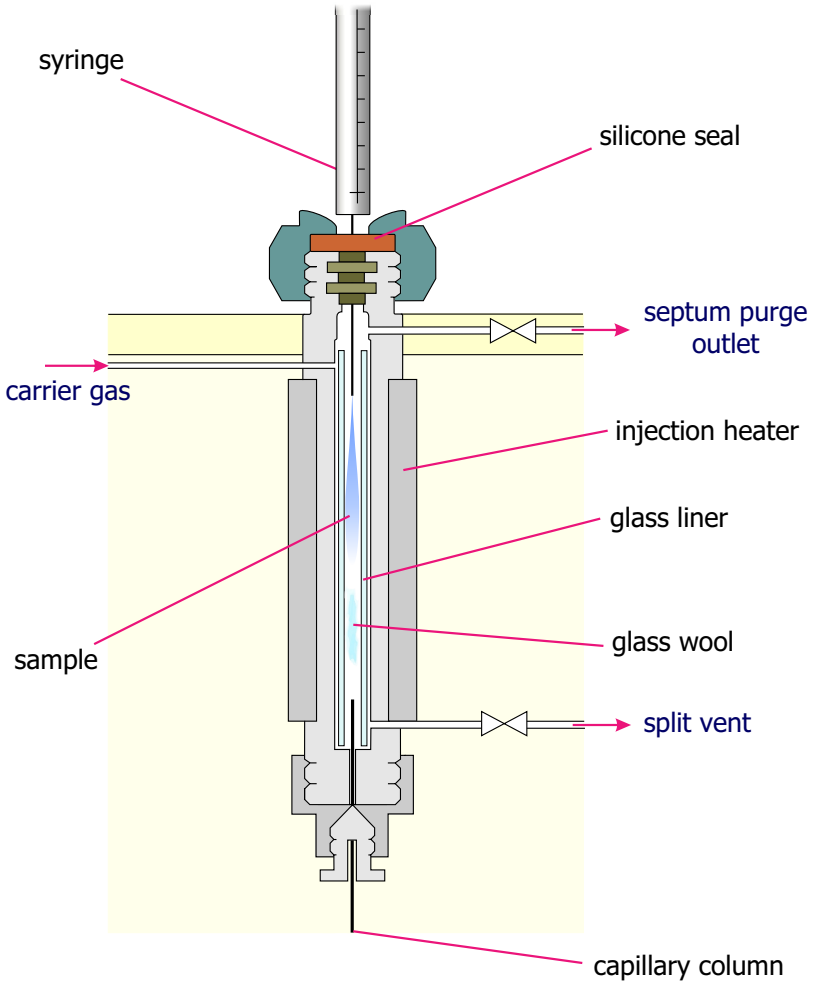


Figure 2.6: Schematic of a typical split/splitless injector adapted from [22].

The other mode of injection is splitless mode. Splitless mode is commonly used in chemical ecology studies because it is more suited to the analysis of trace compounds [3, 8, 25–27]. In this mode the vent valve remains closed for the first 0.5-1.0 minutes so that the entirety of the sample enters the column. Following this the valve opens and any compounds which are not sufficiently volatile and may interfere with the analysis are purged [22]. This mode of operation requires careful consideration of the oven temperature programme to ensure chromatographic efficiency. The use of splitless mode is common within the analysis of cuticular hydrocarbons, because the amounts of hydrocarbons are so small [9, 16, 28–30].

2.3.3.3 Columns

Within the field of gas chromatography there are two types of column; the capillary column and the packed column. Packed columns are far less widely used than capillary columns and have a glass or steel wall along with an inert, stable support, onto which the solid stationary phase is attached. The length of this column is much shorter and the diameter much wider than capillary columns. The performance of this type of column is limited and they are not suitable for trace analyses [22, 24].

Capillary columns are far more suited to the analysis of trace compounds. Capillary columns, also known as open tubular columns, are usually made from high purity, fused silica. To lend flexibility to the column they are coated in polyimide or aluminium. They are usually 12 to 30 m in length, but types exist which are up to 100 m in length. The internal diameter of these columns can range from 100 to 530 μm. The stationary phase in this type of column consists of a thin liquid film which is coated onto the inside of the column, usually a polysiloxane [22, 24]. The film thickness can vary, depending on the type and use of the column, however thicknesses of 0.1 - 5 μm are generally used. The benefit of these types of columns is that they can be used to separate compounds with very low volatilities however this is only possible when the

stationary phase is chemically bonded to the silica column. It should also be noted that due to the very small amount of stationary phase present, these columns are not suitable for bulk separation.

2.3.3.4 Carrier gases

With gas chromatography the mobile phase is a gas, known as the carrier gas. The carrier gas must be inert and free from oxygen and water as these can have a detrimental effect on the stationary phase within the column. The carrier gas does not affect the partitioning mechanism directly, however its viscosity and flow rate can affect the dispersion of analytes within the column, which in turn can affect the efficiency and sensitivity of the detection. Therefore careful consideration should be given to these effects and their results on the analysis. The most common carrier gases are nitrogen, hydrogen and helium, the use of each having advantages and disadvantages [22–24]. Figure 2.7 shows the optimum linear velocity of these three carrier gases.

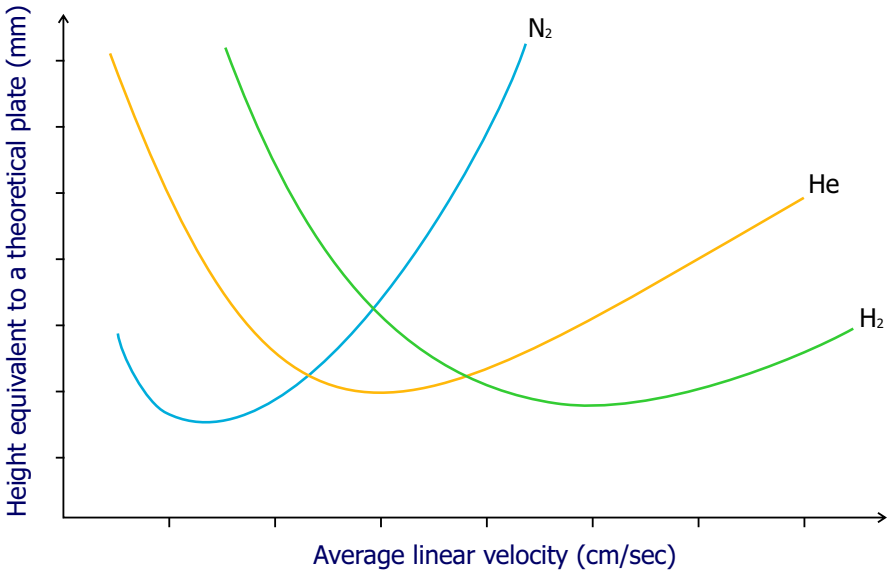


Figure 2.7: Graph showing the optimum linear velocity of carrier gas adapted from [22].

As can be seen from this diagram the efficiency of the separating column (vertical axis) is provided by a concept known as the HETP (height equivalent to a theoretical plate)

value. The HETP value is calculated by dividing the length of column (L), by the number of theoretical plates (N). There are several ways of calculating N, however the most common method involves measuring, from a chromatogram, the retention time of a peak and its width at the point equal to half of its height. As such, the value for HETP is influenced by both the column itself and the solute moving through the system [24]. From Figure 2.7 therefore it can be seen that nitrogen is the most efficient as it has the minimum HETP (height equivalent to a theoretical plate) value, but this efficiency is only obtained at very slow linear velocities, which would mean an extended analysis period. When the linear velocity is increased the efficiency rapidly decreases, as can be seen from the steep upward curve in Figure 2.7. In the same way it can be seen that helium is slightly less efficient than nitrogen but that its maximum efficiency occurs at a higher velocity allowing for a shorter analysis time. The best performance is observed when using hydrogen; this has a minimum HETP value, similar to helium, but this is achieved at a higher velocity, meaning the same separation performance can be achieved at a greater speed and thus a shorter analysis time. Generally most researchers opt for helium due to its ease of use, however with global demand for helium and availability becoming limited, the use of hydrogen is becoming more popular [22–24, 31].

2.3.3.5 Oven

The column sits within a temperature programmable oven, which is used to control the temperature of the column. Within the column the bulk of the separation is achieved using the differences in boiling points of the components within the sample, with the differing affinities to the stationary phase providing extra separation. The oven temperature should generally be kept below the boiling point of the compounds to be separated; this is because if the temperature is much higher than the boiling point then the vapour pressure within the column will be much higher. This will result in the compounds moving through the column too fast and as a consequence there will

be little separation between compounds and poor resolution. However a low column temperature will result in a slower run as the vapour pressure will be lower and the compounds will travel through the column much slower, although they will be highly resolved. The solution is generally a compromise between the two. Within GC analysis there are two ways of programming the oven. A run which holds the GC oven at one temperature throughout the run is known as an isocratic run. This is useful if the compounds within a mix all have similar boiling points [14].

In insect studies a sample will generally contain many compounds with differing boiling points. Therefore for this work a programmed temperature run is more useful. In this mode the temperature of the oven is gradually increased at a specific rate, suitable for the analysis being performed. The rate of temperature change is known as the temperature ramp and during one run several temperature ramps can be used with differing rates. The oven temperature can also be held at a constant temperature at specific points to aid separation. The final oven temperature is typically around 320 °C, regardless of whether this is required for separation. This is to ensure that all compounds move through the system to prevent carry over. There are however several disadvantages of this mode. After each run the instrument is required to cool back down to its starting temperature, depending on the ambient temperature and the temperature to be achieved, this process can take several minutes adding to the overall run time. Additionally programmed temperature runs tend to have noisier baselines at higher temperatures, which can obscure the signals of less concentrated compounds [14]. For a schematic showing a typical temperature programme see Figure 2.8.

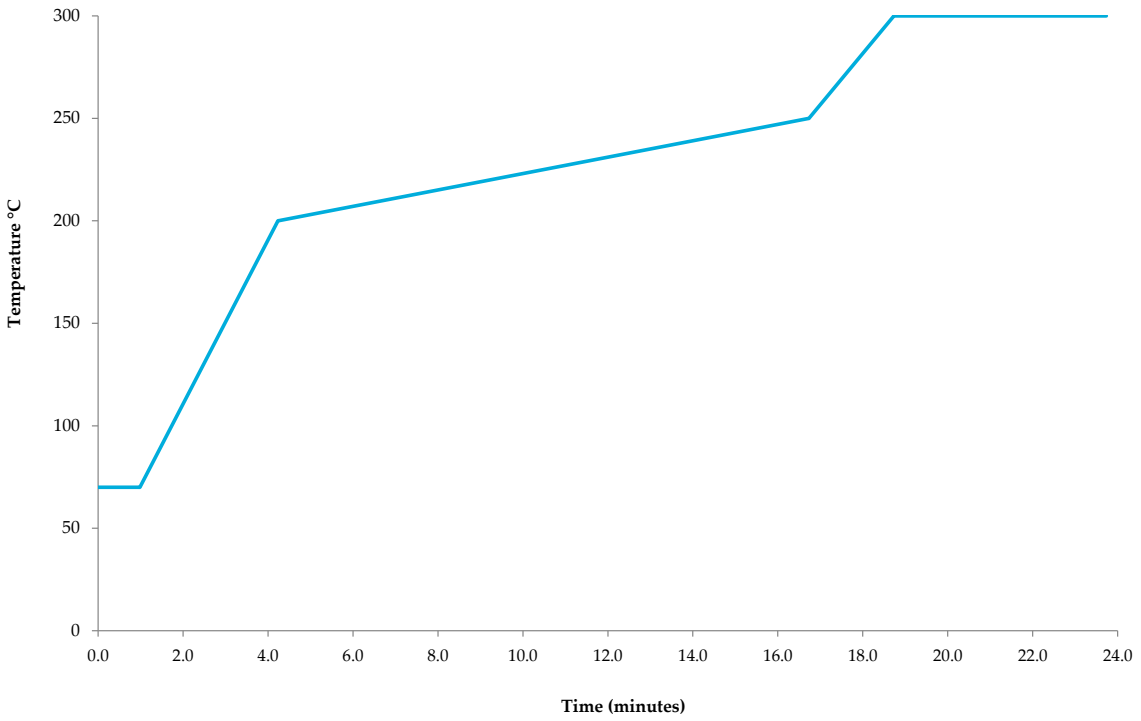


Figure 2.8: A schematic showing a typical temperature programme for a GC oven. The gradient of the blue line indicates the temperature ramp; many different ramps can be used to achieve effective separation.

2.3.3.6 Detectors

The final component of a GC system is the detector. The role of the detector is to produce an electrical signal which is proportional to the concentration of the analyte. There are many detectors available for gas chromatographs, some of which involve the coupling of a spectroscopic instrument to provide subsequent identification of the analytes [24]. The most common non-spectroscopic detector is the flame ionisation detector. The FID is a universal detector as it will provide a response to nearly all organic compounds, with the exception of CO, CO_2 and HCN [24]. Upon exiting the column the gas flow passes through a small burner which combusts the organic components within the sample. For this reason there needs to be a separate hydrogen and oxygen supply to this part of the instrument regardless of whether hydrogen is used as the carrier gas. The combustion of a molecule containing carbon creates positively charged particles that generate a small current between two electrodes [22]. The benefit of this type of detector is that despite the ionisation efficiency being low, the signal to noise

ratio is very high, which results in very high sensitivity (near ppb levels for 1 μL of sample injected) and dynamic range [24]. The drawback to this type of detector is that it will not identify the compounds present. In order to identify the compounds a series of standards of the suspected compounds must be run to see if the retention times match. This clearly suggests that some idea of a compound's identity must be known and therefore this detector is unsuitable for complex samples which may contain many unknown compounds.

2.3.4 Mass spectrometry

The most widely employed hyphenated technique for GC systems is gas chromatography-mass spectrometry (GC-MS), that is the separating power of a gas chromatograph coupled to the qualitative power of a mass spectrometer detector. This technique is widely used within ecological, environmental, pharmaceutical, and petroleum based studies [32–35]. As with other detectors, there are many sub types of mass spectrometer, however they all rely on the ionisation of the analyte components.

2.3.4.1 Compound ionisation

There are two main types of ionisation used within GC-MS. These are electron ionisation and chemical ionisation. Chemical ionisation (CI) is less common and is considered to be a soft ionisation source in that it generally results in less fragmentation of the analyte molecules and thus a clearer indication of the unknown compound's molecular weight. In CI a large amount of reagent gas (methane, isobutane or ammonia) is first ionised via electron bombardment. This ionised reagent gas then in turn ionises the analyte compounds via collision and results in both positively and negatively charged species [22, 24].

In contrast, electron ionisation (EI) is the more widely used method and is considered

a hard ionisation source, leading to highly reproducible spectra which can be compared to a library of references. With this technique high energy electrons are emitted by a heated tungsten filament. These electrons are accelerated using a potential of typically 50-100 eV, however 70 eV is most commonly used as it results in highly reproducible spectra [22,24]. When the gaseous analyte molecules collide with this high energy electron stream it causes the analytes to lose electrons and fragment [14], see Equation 2.1.

$$M + e^- \rightarrow M^+ + 2e^- \tag{2.1}$$

It should be noted however that rarely does physical impact occur, instead ionisation and fragmentation are induced when an electron passes closely to an analyte molecule. This causes a large fluctuation in the electric field and thus ionisation and fragmentation [36]. However because the ionisation mechanism is still dependant on very close proximity between the electrons and the analytes the ionisation rate is low, sources reporting it to be between 1 in 10,000 and 1 in 1,000,000 [22, 24]. The resulting fragmentation however is extensive with the molecular ion making up only a small proportion of detectable ions. Following ionisation the charged molecules are repelled using a positively charged plate (the ion repeller) into the mass analyser. Here the ionic particles are separated according to their mass-to-charge ratio *(m/z)*. There are various types of mass analysers that can be coupled to GC instruments, however this thesis will discuss only the type used within this project.

2.3.4.2 Quadrupole mass analysers

Quadrupole mass analyser systems are the most commonly used due to their cost and resolution. A quadrupole has four hyperbolic rods running parallel to each other. To these rods direct current and radio frequency potentials are applied so that each pair of opposite rods has the same charge, whilst the neighbouring rods have a different charge. These potentials are rapidly reversed, see Figure 2.9.

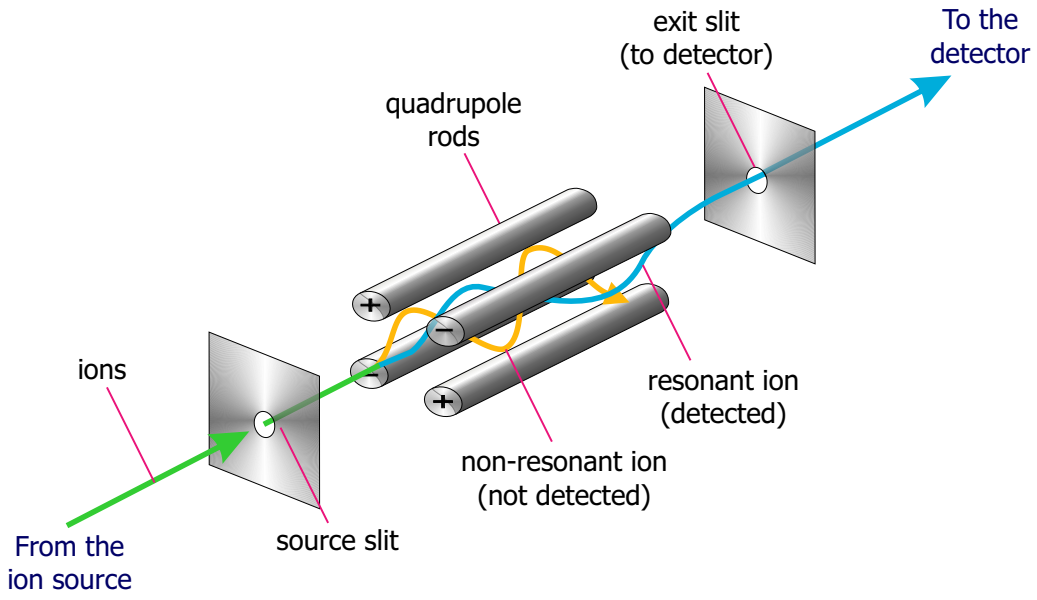


Figure 2.9: The basic setup of a quadrupole mass analyser.

The ions traverse the space in between the rods due to the potentials applied. However by varying the ratio of the potentials, instability in the electric field can be introduced. This instability prevents any fragments with certain mass-to-charge ratios from travelling the length of the mass analyser. Other fragments are deflected into the rods and do not reach the detector. In this way the instrument can scan through the desired range and measure the amount of each fragment present [14]. This is known as scan mode. The other mode is selected ion monitoring, or SIM mode. This is more useful when the analyst is only interested in a certain ion mass. This mode also has the added advantage of greatly reducing the background signal, known as the baseline, and thus increases the sensitivity of the instrument [19].

2.3.5 Gas chromatography-mass spectrometry

Despite the various instrumental combinations available, the most common for the analysis of hydrocarbons is gas chromatography coupled to a quadrupole mass spectrometer. This technique utilises the separating power of gas chromatography with

the identification power of a mass spectrometry detector. Quadrupole GC-MS systems are, compared to other instruments, fairly low-cost, robust and compact. They are therefore extremely widely used within the field of chemical ecology for the analysis of a variety of samples and shall be used throughout this project [27, 28, 30, 39–41].

2.3.6 Other techniques

Although GC-MS is the most commonly used technique in studies of this type, it is by no means the only available technique for the analysis of cuticular hydrocarbons. Other techniques that are used include Matrix-assisted Laser Desorption/Ionisation (MALDI) [42]. This is a different type of mass spectrometry and often MALDI systems are connected to Time-of-Flight (ToF) mass analysers. One relevant study that utilised MALDI-ToF was that by Cvačka et al. in 2006 [42]. This study looked at the CHCs present on several different insect species and compared the results obtained to those using GC-MS.

The insects were prepared using whole body extracts in chloroform and the hydrocarbon fraction was separated from the rest of the extract using thin layer chromatography. Following this each spot was scraped off the plate, and analysed using a MALDI-ToF instrument. The results showed that although GC-MS was able to detect the lower molecular weight compounds and showed good correlation with the MALDI-ToF, the latter technique detected a greater abundance of ions between m/z 500 and 700. There was also a second series of hydrocarbons at m/z 800-850; the largest hydrocarbons had more than 70 carbon atoms present. These were thought to be cyclised compounds or long chain hydrocarbons with multiple sites of unsaturation [42].

It is worth mentioning however that there are GC-MS systems that are capable of high temperature analysis, such at that used by Akino in 2006 to analyse the hydrocarbons of *Formica truncorum* [43]. In this study the authors detected CHCs containing up

to 48 carbons. Whilst there are definite advantages to using systems such as MALDI, there are also drawbacks. Firstly the sample preparation is slower and more complicated, and secondly analysis of the hydrocarbons is more complex, as there is no compound separation.

The final technique is one which is becoming more prevalent in the analysis of insect hydrocarbons; Direct Analysis in Real Time-Mass Spectrometry (DART-MS). Whole body liquid extracts are fatal to insects and whilst SPME offers a viable non-lethal alternative the sensitivity and extraction procedure are less than ideal. In a study by Yew et al. in 2008 the authors investigated the use of DART-MS as a non-lethal alternative for the analysis of *Drosophila melanogaster* CHCs [44]. They first exposed the fly directly to the heated helium metastable stream. However they found that although they observed signals that matched those found using GC-MS this caused severe, and often lethal trauma to the insect. As the primary driving force behind the use of this technique was its non-lethal nature this was a problem that had to be addressed. They determined that high quality results could also be achieved by repeated contact with the insect using a metal probe. This was then in turn sampled under the helium stream [44].

However, whilst this is a viable alternative to GC-MS, it does have drawbacks. This technique is more labour intensive; whilst analysis time is rapid, there is the extra sample preparation time and each sample must be run manually. In addition the compounds within the sample are also not separated therefore analysis is much more complex. With GC-MS however, multiple samples can be run without any input from the analyst and the analysis of the resulting data is relatively straightforward.

2.4 Compound Identification

2.4.1 Overview

Following the chromatographic separation of the complex mixture of cuticular hydrocarbons by the GC, each compound must be identified by the resulting mass spectrum. Whilst this can, to some extent, be achieved by comparison to reference spectra within a database, often a particular compound requires a process of manual identification. This is because a reference spectrum for the exact compound is not present within the database due to the large number of isomers of a particular compound.

2.4.2 Characteristic ions

Whilst the identification of alkenes and alkanes from their mass spectra is relatively straightforward, the identification of methyl and dimethyl compounds is more complex as similar branched compounds can co-elute, which leads to complicated fragmentation patterns. The identification of these branched compounds occurs by identifying characteristic ions and using these to elucidate the compounds present. These characteristic ions can be used to firstly determine the number of compounds co-eluting and subsequently their identities. Figure 2.10 shows a chromatogram representing the CHC profile of a *Myrmica sabuleti* ant.

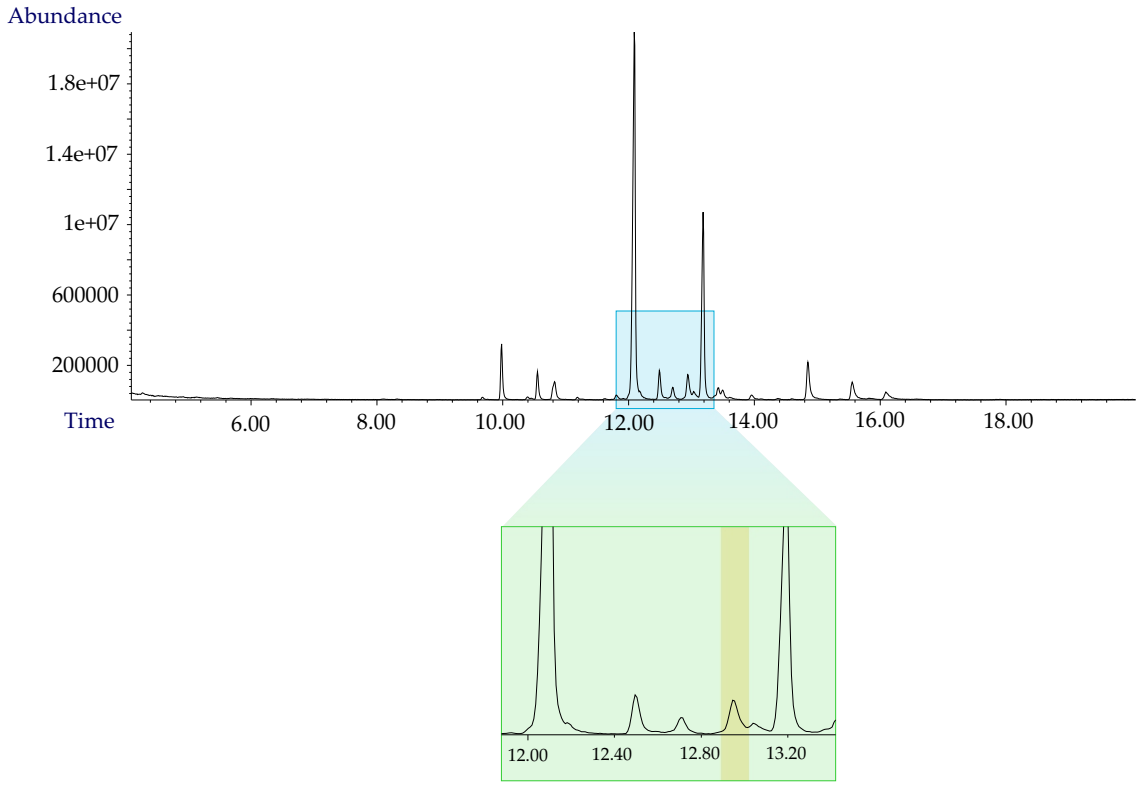


Figure 2.10: The modified chromatogram above shows an enlarged section of the trace, with the yellow bar indicating the peak of interest. From its shape this peak appears to represent a single compound.

The yellow bar shown in Figure 2.10 indicates a seemingly single peak within this chromatogram, however the mass spectrum shown in Figure 2.11 which corresponds to the maximum of this peak reveals that there are two monomethyl compounds co-eluting. This can be seen by looking at the fragments shown within the mass spectrum. Studying the mass spectrum shown in Figure 2.11 reveals that there are four fragments with even masses (circled). The appearance within a mass spectrum of even numbered mass fragments reveals that there is a branched compound indicated as even fragments are not found in the mass spectra of straight chain compounds. Once all the relevant fragments are identified they must be correctly paired up. Finally the position of the methyl-branch can be determined by looking at the size of the smaller fragment within each characteristic ion pair.

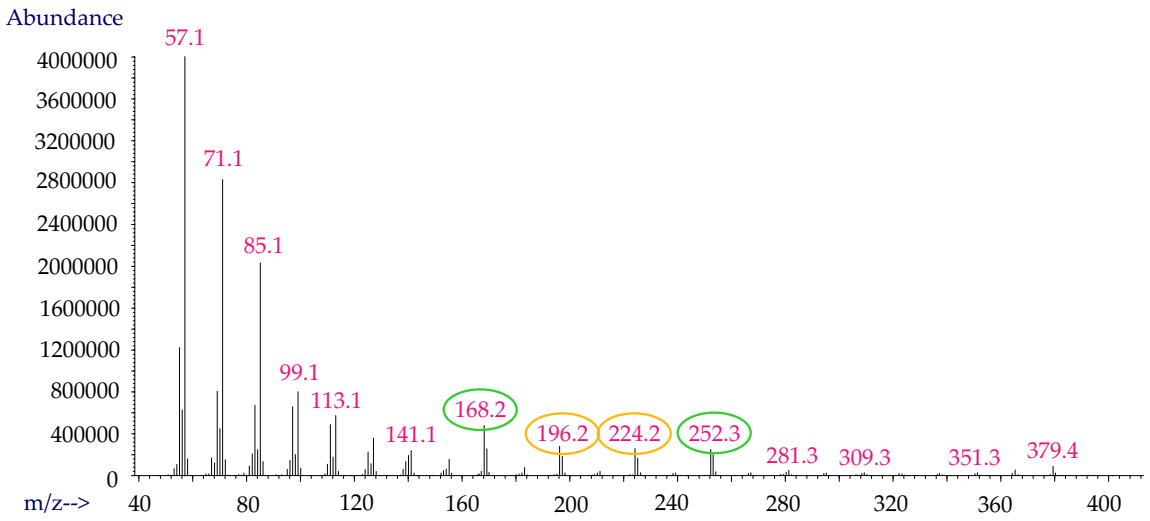


Figure 2.11: A mixture of 11-methyl (green) and 13-methylheptacosane (yellow) identified using the characteristic ions of m/z 168, 196, 224, and 252.

Figure 2.12 shows how the two methyl-branched compounds fragment within the Mass Analyser. As it can be seen, each methyl-branched compound undergoes alpha cleavage around the methyl branch. However the position of the bond that cleaves can be either of those around the methyl branch. Each cleavage, one shown in pink and the other blue, results in a corresponding charged species and radical species. Only the charged species are detected by the quadrupole detector. Using this schematic it can be seen that a mixture of 11-methyl, and 13-methylheptacosane produces four charged species of differing masses as shown in Figure 2.11.

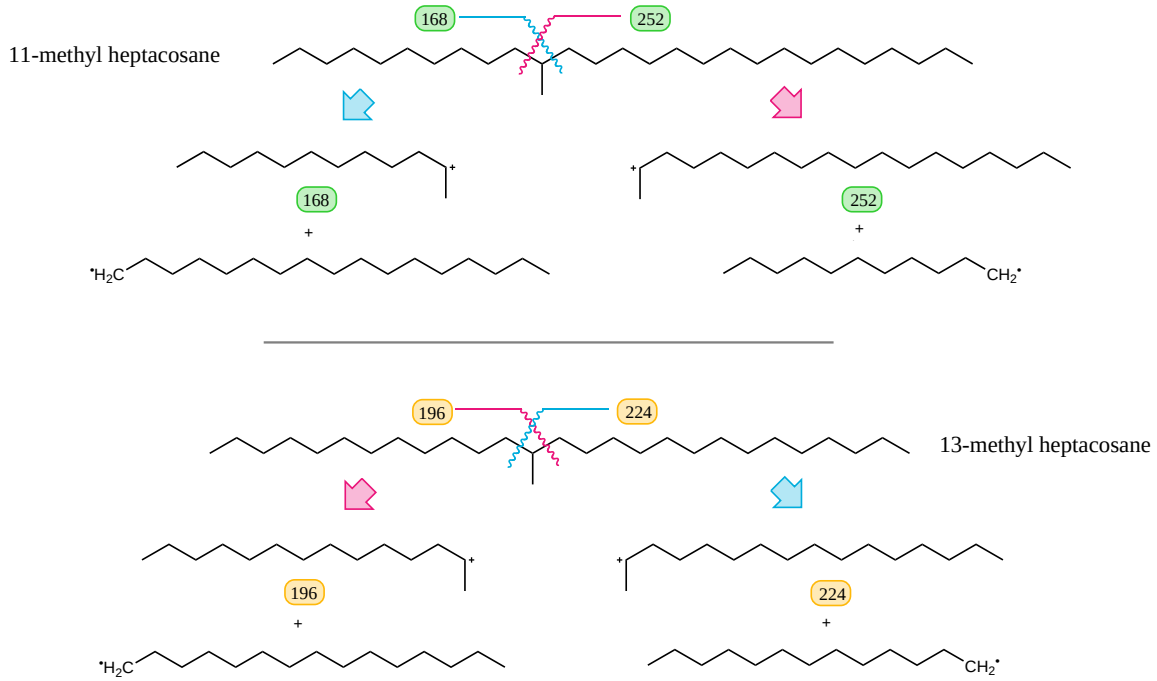


Figure 2.12: Schematic indicating the fragmentation mechanisms of a mix of two monomethyl alkanes and the subsequent formation of their charateristic ions.

Whilst a change in column or adjustments to the method can help with the chromatographic resolution, successful compound identification is still challenging, and often depends on method optimisation and practice.

2.4.3 Kovats indices

Another method used to aid identification of branched hydrocarbons is the Kovats index system developed in the 1950s by Ervin Kováts, see Equation 2.2.

$$I = 100 \times \left[n + (N - n) \frac{t_{\mathrm{r(unknown)}} - t_{\mathrm{r(n)}}}{t_{\mathrm{r(N)}} - t_{\mathrm{r(n)}}} \right] \tag{2.2}$$

In this equation I represents the Kovats retention index (KI), n is the number of carbon atoms in the smaller n-alkane, N is the number of carbon atoms in the larger n-alkane, and t_r is the retention time. This system uses the known retention times of the alkanes eluting immediately before and after the unknown compound, in this equation these would be n and N respectively. These values are input into this mathematical formula along with the retention time of the unknown compound. The equation normalises the retention time of the unknown methyl-branched hydrocarbon to the input straight chain alkane retention times and provides a resulting index value which relates to the number of carbons within the unknown methyl-branched hydrocarbon. This technique is widely used within many different studies which rely on the accurate identification of cuticular hydrocarbons [3, 4, 30, 45–47]. The main benefit of this system is that the retention time of the compounds are converted from system dependent values into independent constants. These constants can then be used with a table of elution patterns and KI values, thus assisting the identification of monomethyl, dimethyl and trimethyl alkanes [48].

2.4.4 Derivatisation

Within chemical ecology studies the technique of sample derivatisation can be used to facilitate the identification of unsaturated compounds. Although many species of ants produce unsaturated compounds, the position of the double bond can vary. The most commonly encountered alkene is the Z9 isomer [49], although other positions are also found [27, 49]. Therefore it is useful to have a procedure to determine the isomeric position of the double bond. This is most commonly achieved via a dimethyl disulfide (DMDS) reaction. This reaction involves the addition of two CH_3S groups across the double bond. When the molecule undergoes fragmentation the double bond position is easily identified since cleavage primarily occurs at the carbon-carbon bond adjacent to those with the CH_3S groups, leading to two main diagnostic ions, represented by $A^{(+)}$ and $B^{(+)}$ [50]. See Figure 2.13.

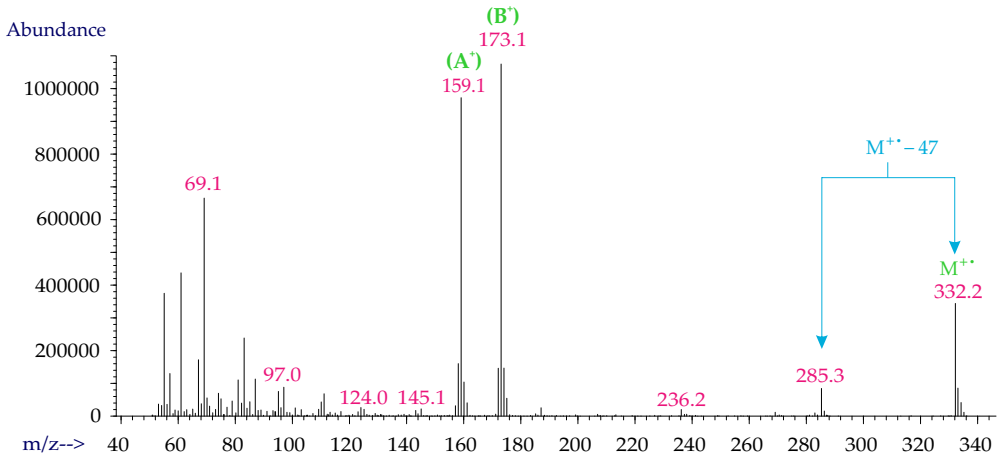


Figure 2.13: A schematic of the DMDS reaction, based on [50], here it can be seen that the addition of two CH_3S groups and subsequent fragmentation leads to two diagnostic ions, $A^{(+)}$ and $B^{(+)}$.

This fragmentation leads to characteristic mass spectra, the interpretation of which provides the double bond positions, see Figure 2.14. For a more detailed explanation of the identification of double bond position following a DMDS reaction see Section 3.3.2.

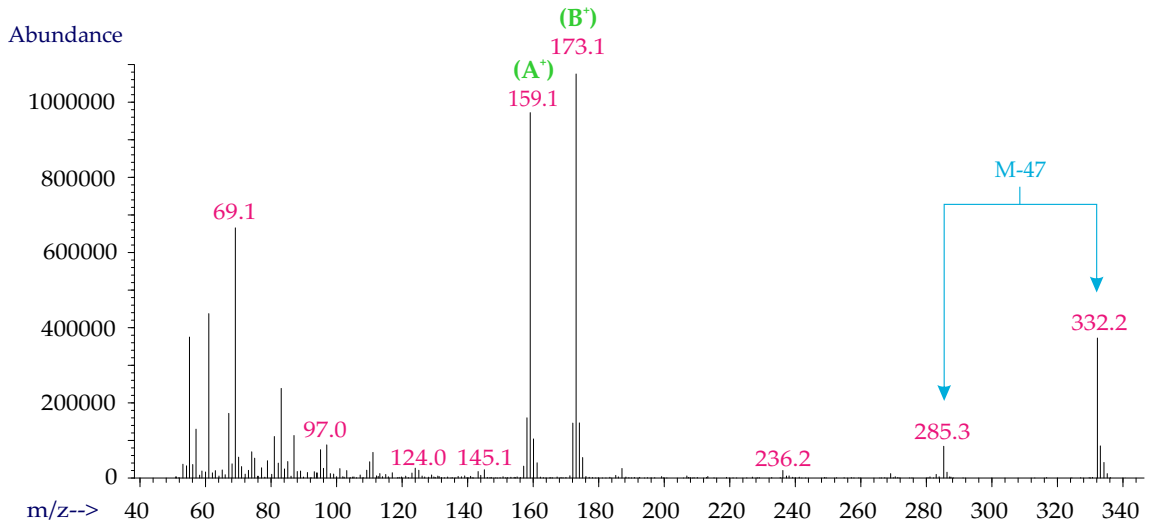


Figure 2.14: The resulting DMDS mass spectrum. The masses of $A^{(+)}$ and $B^{(+)}$ equal the molecular weight of the derivatised compound, in this case heptadecene (m/z 332) whilst the masses of the fragments indicate the double bond position (Z8). The peak at m/z 285 is due to the loss of a SMe group from the derivatised, fragmented product.

2.5 Statistical Analysis

2.5.1 Overview

When performing any analytical technique often large amounts of data are produced. Particularly in this project there was a need to be able to compare the results of one population against another. Therefore in order to determine whether one population is statistically significantly different from the other a suitable numerical test is required. There are many tests that can be performed to quickly identify significance between populations, for example the t-test. However not all statistical techniques are suitable for every data set. Often there are certain characteristics of particular data sets that mean that some tests are invalid, or would provide misleading results if used [51]. For example some tests require data to be normally distributed; other tests may rely on the populations being dependant upon each other. Finally there are some tests that can only be used with two populations whilst some can be used to compare more than two populations, for example the ANOVA test.

2.5.2 Non-parametric ranking

Many statistical techniques require the data to demonstrate a normal distribution. Normal distributions are found throughout nature and can be represented by the profile shown in Figure 2.15. As shown in this figure, the x axis represents the value which is measured and the y axis the frequency with which that value occurs. The symmetrical curve shows that the closer to the mean a value falls, the greater its frequency [51,52].

The main issue surrounding the analysis of the data in this project was the large variance surrounding the numbers in the raw data set. The large variation within the raw data meant that the distribution profiles were not normal, and therefore a technique suitable for use with non-normalised data was necessary. Such techniques are known as non-parametric and make no assumptions about the distribution pro-

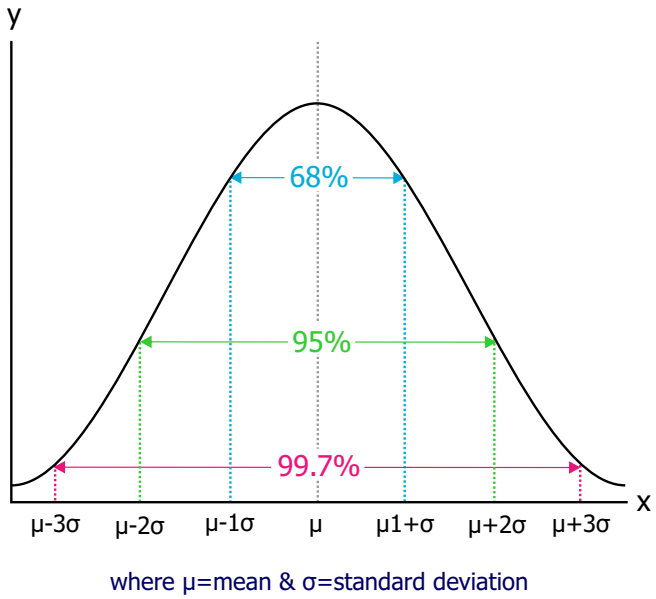


Figure 2.15: Properties of a normal distribution curve where approximately 68% of values fall within +/- 1 standard deviation of the mean; approximately 95% of values fall within +/- 2 standard deviations of the mean and approximately 99.7% of values fall within +/- 3 standard deviations of the mean, adapted from [51].

file of the data [52]. Whilst there are many non-parametric calculations that can be performed it was necessary to use one that compares two independent samples, such as the Mann-Whitney U-test. The Mann-Whitney U-test is widely used in biological studies particularly when testing whether a group of experimentally treated organisms is different to the controls. The test works by ranking the raw data in terms of the size of the values, thus removing any large variances. Any statistical differences between the two populations are therefore based on the results of the ranking, rather than the raw data itself [52].

Although the Mann-Whitney U-test can be calculated by hand, it is more common to use a statistical computer software package such as Minitab or SPSS. For the duration of this project SPSS (IBM, US) was the statistical package of choice. Using this software the values associated with the groups to be compared are input and defined. First the values are pooled and ranked with the smallest value assigned a rank of 1; the sum of the ranks and mean ranks are also calculated. Next the total rank of each group is

determined by separately summing the individual ranks associated with each group to give the values known as R_1 and R_2. The final step is to calculate the test statistics of U_1 and U_2 and the lower of the two values is taken as the Mann-Whitney U value. The equations used to calculate U_1 and U_2 are shown in Equations 2.3 and 2.4. The parameters n_1 and n_2 are the sample sizes of group 1 and 2 respectively [52].

$$U_1 = n_1 n_2 + \frac{n_2(n_2+1)}{2} - R_2 \tag{2.3}$$

$$U_2 = n_1 n_2 + \frac{n_1(n_1+1)}{2} - R_1 \tag{2.4}$$

It is worth mentioning here that in many cases one group may have a much higher set of ranks than the other group. In this instance one value of U will be large and the other will be small. Whilst the two values for U can be similar or very different the smaller of the two is always that which is compared to a statistical table. There are two types of statistical table which can be used. If the group sizes are 20 or less, i.e. n_1 and $n_2 \leq 20$, then a critical values table for Mann-Whitney tests can be used. If n is greater than 20, then a Z-test table can be used instead. If the Mann-Whitney U value is less than or equal to the critical value then the null hypothesis is rejected, i.e. the two populations are different to each other. Rather than simply comparing the two values a software package also calculates the probability that the Mann-Whitney U value would be less than or equal to the critical value if the samples were not statistically significant [51, 52]. This probability value is known as the p value. In this project all probability values are based on one-tailed tests. This is because incorporation of the labelled substrate would cause an increase in the percentage abundance values for the substrate group. However no incorporation would not cause a decrease in percentage abundances. As the expected change was in one direction only a one tailed test was chosen [51, 52]. Further information on the use of this statistical procedure is provided in Section 3.4.2.2.

2.5.2.1 Bonferroni Correction

The Bonferroni correction is a simple post hoc statistical test which is used to reduce the chance of a Type I error [53]. Simply put, a Type I error is a false positive, i.e. significance is indicated when there is none and the null hypothesis is wrongly rejected [52]. The chances of a Type I error occuring are much greater when many statistical tests are performed at the same time, in this instance the use of a Bonferroni correction can be prudent to remove the risk of a Type 1 error occuring. When used, the Bonferroni correction adjusts the probability value, p, and makes it more conservative, thus reducing the risk of false positives [53]. In turn however the use of this correction can lead to statisical significance being overlooked, as the required p value for significance is much smaller than what it would be without this correction. For this reason it was decided that the statistical analysis within this thesis would not be subjected to a Bonferroni correction, as this post hoc adjustment would result in less sensitivity of the statistical methods and thus some important minor incorporation could be missed.

References

[1] Stanley-Samuelson DW, and Nelson DR, *Insect Lipids: Chemistry, Biochemistry, and Biology*, (University of Nebraska Press, 1993)

[2] Van Zweden JS, Brask JB, Christensen JH, Boomsma JJ, Linksvayer TA, and D'Ettore P, 'Blending of heritable recognition cues among ant nestmates creates distinct colony gestalt odours but prevents within-colony nepotism,' (2010), *J. Evol. Biol.*, 23, 1498-1508

[3] Moore HE, Adam CD, and Drijfhout FP, 'Potential use of hydrocarbons for aging *Lucilia sericata* blowfly larvae to establish the postmortem interval,' (2012), *J. Forensic Sci.*, 58, 1-9

[4] Martin SJ, Shemilt S, and Trontti K, 'Nest-mate recognition cues are not used during or influenced by mating in the ant *Formica exsecta*,' (2013), *Ethol. Ecol. Evol.*, 26, 40-48

[5] Schroeder FC, Gonzàlez A, Eisner T, and Meinwald J, 'Miriamin, a defensive diterpene from the eggs of a land slug (*Arion* sp.),' (1999), *Proc. Natl. Acad. Scie. U.S.A.*, 96(24), 13620-13625

[6] Wagner D, Tissot M, Cuevas W, and Gordon DM, 'Harvester ants utilize cuticular hydrocarbons in nestmate recognition,' (2000), *J. Chem. Ecol.*, 26(10), 2245-2257

[7] Hay-Roe MM, Lama G, and Nation JL, 'Pre- and postzygotic isolation and Haldane rule effects in reciprocal crosses of *Danaus erippus* and *Danaus plexippus* (Lepidoptera: Danainae), supported by differentiation of cuticular hydrocarbons, establish their status as separate species,' (2007), *Biol. J. Linn. Soc.*, 91, 445-453

[8] Mullen SP, Mendelson TC, Schal C, and Shaw KL, 'Rapid evolution of cuticular hydrocarbons in a species radiation of acoustically diverse Hawaiian crickets (Gryllidae: Trigonidiinae: *Laupala*),' (2007), *Evolution*, 61(1), 223-231

[9] Martin SJ, Takahashi JI, Ono M, and Drijfhout FP, 'Is the social parasite *Vespa dybowskii* using chemical transparency to get her eggs accepted,' (2008), *J. Insect Physiol.*, 54(4), 700-707

[10] Vander Meer R, Saliwanchik D, and Lavine B, 'Temporal changes in colony cuticular hydrocarbon patterns of *Solenopsis invicta*,' (1989), *J. Chem. Ecol.*, 15(7), 2115-2125

[11] Morgan ED, and Wadhams LJ, 'Gas chromatography of volatile compounds in small samples of biological materials,' (1972), *J. Chromatogr Sci.*, 10(8), 528-529

[12] Bagnères AG, and Morgan ED, 'A simple method for analysis of insect cuticular hydrocarbons,' (1990), *J. Chem. Ecol.*, 16(12), 3263-3276

[13] Vienne C, Bagnères AG, Errard C, and Lange C, 'Etude chimique de la reconnaissance interindividuelle chez *Myrmica rubra* et *Manica rubida* (Formicidae, Myrmicinae) élevées en colonies mixtes artificielles,' (1990), Act. Coll. Insectes Soc., 6, 261-265

[14] McNair HM, and Miller JM, *Basic Gas Chromatography*, (John Wiley and Sons Inc., 1998)

[15] Millar JG, and Haynes KF, *Methods in Chemical Ecology*, (Kluwer Academic Publishers, 1998)

[16] Monnin T, Malosse C, and Peeters C, 'Solid-phase microextraction and cuticular hydrocarbon differences related to reproductive activity in queenless ant *Dinoponera quadriceps*,' (1998), *J. Chem. Ecol.*, 24(3), 473-490

[17] Steffen A, and Pawliszyn J, 'Analysis of flavor volatiles using headspace solid-phase microextraction,' (1996), *J. Agric. Food Chem.*, 44(8), 21872193

[18] Belliardo F, Bicchi C, Cordero C, Liberto E, Rubiolo P, and Sgorbini B, 'Headspace-solid-phase microextraction in the analysis of the volatile fraction of aromatic and medicinal plants,' (2006), *J. Chrom. Sci.*, 44(7), 416-429

[19] Langford A, Dean J, Reed R, Holmes D, Weyers J, and Jones A, *Practical Skills in Forensic Science*, (Pearson Education Limited, 2005)

[20] Coskun O, 'Separation techniques: Chromatography,' (2016), *North Clin. Istanb* 3(2), 156-160

[21] Meyer VR, *Practical High-Performance Liquid Chromatography, 5th Edition*, (Wiley, 2010)

[22] Rouessac F, and Rouessac A, *Chemical Analysis Modern Instrumentation Methods and Techniques* (John Wiley and Sons Inc., 2000)

[23] Miller JM, *Chromatography Concepts and Contrasts*, (John Wiley and Sons Inc., 2005)

[24] Schomburg G, *Gas Chromatography A Practical Course*, (VCH, 1990)

[25] El-Showk S, van Zweden JS, D'Ettorre P, and Sundström L, 'Are you my mother? Kin recognition in the ant *Formica fusca*,' (2010), *J. Evol. Biol.*, 23(2), 397-406

[26] Howard RW, Pérez-Lachaud G, and Lachaud J-P, 'Cuticular hydrocarbons of *Kapala sulcifacies* (Hymenoptera: Eucharitidae) and its host, the Ponerine ant *Ectatomma ruidam* (Hymenoptera: Formicidae),' (1994), *Ann. Entomol. Soc. Am.*, 94(5), 707-716

[27] Guillem RM, Drijfhout FP, and Martin SJ, 'Using chemo-taxonomy of host ants to help conserve the large blue butterfly,' (2012), *Biol. Conserv.*, 148(1), 39-43

[28] Akino T, and Yamaoka R, 'Chemical mimicry in the root aphid parasitoid *Paralipsis eikoae* Yasumatsu (Hymenoptera: Aphidiidae) of the aphid-attending ant *Lasius sakagamii* Yamauchi & Hayashida (Hymenoptera: Formicidae),' (1998), *Chemoecology*, 8(4), 153-161

[29] Martin SJ, Carruthers JM, Williams PH, and Drijfhout FP, 'Host specific social parasites *(Psithyrus)* indicate chemical recognition system in bumblebees,' (2010), *J. Chem. Ecol.*, 36(8) 855-865

[30] Nation JL, Sandford MT, and Milne K, 'Cuticular hydrocarbons from *Varroa jacobsoni*,' (1992), *Exp. Appl. Acarol.*, 16, 331-344

[31] Robinson JW, Skelly Frame EM, and Frame II GM, *Undergraduate Instrumental Analysis*, (CRC Press, 2014)

[32] Petruzzi J, 'GC/MS in drug analysis,' (1973), *Anal. Chem.*, 45(14), 1213

[33] Eckenrode BA, 'Environmental and forensic applications of field-portable GC-MS: An overview,' (2001), *J. Am. Mass Spectrom.*, 12(1), 683-693

[34] Pavlova A, and Papazove D, 'Oil-spill identification by gas chromatography-mass spectrometry,' (2003), *J. Chrom. Soc.*, 41, 271-273

[35] Goodwin TE, Eggert MS, House SJ, Weddell ME, Schulte BA, and Rasmussen LEL, 'Insect pheromones and precursors in female african elephant urine,' (2006), *J. Chem. Ecol.*, 32, 1849-1853

[36] de Hoffmann E, and Stroobant V, *Mass Spectrometry Principles and Applications, 2nd Edition*, (John Wiley and Sons Inc., 2001)

[37] Chernushevich IV, Loboda AV, and Thomson BA, 'An introduction to quadrupole-time-flight mass spectrometry,' (2001), *J. Mass. Spectrom.*, 36, 849-865

[38] Rubinson KA, and Rubinson JF, *Contemporary Instrumental Analysis*, (Prentice Hall, 2000)

[39] Nelson DR, and Charlet LD, 'Cuticular hydrocarbons of the sunflower beetle, *Zygogramma exclamationis*,' (2003), *Comp. Biochem. Physiol. Part B: Biochem. Mol Biol.*, 135, 273-284

[40] Thomas ML, and Simmons LW, 'Sexual dimorphism in cuticular hydrocarbons of the Australian field cricket *Teleogryllus oceanicus* (Orthoptera: Gryllidae),' (2008), *Insect Physiol.*, 54(6), 1081-1089

[41] Devigne C, and de Biseau J-C, 'The differential response of workers and queens of the ant *Lasius niger* to an environment marked by workers: Ants dislike the unknown,' (2012), *Behavioural Processes*, 91(3), 275-281

[42] Cvačka J, Jiroš P, Sobotník J, Hanus R, and Švatoš A, 'Analysis of insect cuticular hydrocarbons using matrix-assisted laser desorption/ionization mass spectrometry,' (2006), *Chem. Ecol.*, 32(2), 409-434

[43] Akino T, 'Cuticular Hydrocarbons of *Formica truncorum* (Hymenoptera: Formicidae): Description of new very long chained hydrocarbon components,' (2006), *Appl. Entomol. Zool.*, 41(4), 667-677

[44] Yew JY, Cody RB, and Kravitz EA, 'Cuticular hydrocarbon analysis of an awake behaving fly using direct analysis in real-time time-of-flight mass spectrometry,' (2008), *Proc. Natl. Acad. Sci. U.S.A.*, 105(20), 7135-7140

[45] Lommelen E, Johnson CA, Drijfhout FP, Billen J, Wenseleers T, and Gobin B, 'Cuticular hydrocarbons provide reliable cues of fertility in the ant *Gnamptogenys striatula*,' (2006), *J. Chem. Ecol.*, 32(9), 2023-2034

[46] Bernier UR, Carlson DA, and Geden CJ 'Gas chromatography/mass spectrometry analysis of the cuticular hydrocarbons from parasitic wasps of the genus *Muscidifurax*,' (1998), *J. Am. Soc. Mass Spectrom.*, 9(97), 320-332

[47] Calderón-Fernández GM, and Juárez MP, 'The cuticular hydrocarbons of the *Triatoma sordida* species subcomplex (Hemiptera: Reduviidae),' (2013), *Mem. Inst. Oswaldo Cruz*, 108(6) 778-784

[48] Carlson DA, Bernier UR, and Sutton B, 'Elution patterns from capillary GC for methyl-branched alkanes,' (1998), *J. Chem. Ecol.*, 24(11), 1845-1865

[49] Martin S, and Drijfhout F, 'A review of ant cuticular hydrocarbons,' (2009), *J. Chem. Ecol.*, 35(10), 1151-1161

[50] Carlson DA, Roan CS, Yost RA, and Hector J, 'Dimethyl disulfide derivatives of long chain alkenes, alkadienes and alkatrienes for gas chromatography/mass spectrometry,' (1989), *Anal. Chem.*, 61(14), 1564-1571

[51] Miller JN, and Miller JC, *Statistics and Chemometrics for Analytical Chemistry, 6th Edition*, (Prentice Hall, 2010)

[52] Ennos R, *Statistical and Data Handling Skills in Biology, 3rd Edition*, (Pearson, 2012)

[53] Armstrong R, 'When to use the Bonferroni correction,' (2014), *Ophthalmic Physiol. Opt.*, 34, 502-508

Chapter 3

Experimental Methods

3.1 Study Species Husbandry

Throughout this project all ant species were collected from the wild as fragments of established colonies within the Peak District, located in central Britain. These colony fragments were replenished periodically as and when required, although typically every spring. The various *Myrmica* species were collected at the genus level at random due to the morphologically similar appearance of the species. However the other species collected in this study were positively identified based on location and appearance prior to their collection. Colony fragments were transferred into specialised perspex containers or arenas, measuring 14 x 15 x 29 cm. These were obtained from an online retailer for ant supplies (Queen Ant Shop, London), since out of business. Each container had a sliding glass lid with a ventilation hole which was covered with a very fine mesh. Each arena was connected via tubing to a small plastic tub which served as a foraging area. Each of the eight arenas were kept at room temperature (approximately 18-23 °C). For all experimental work smaller plastic containers were used, similar to those used for the foraging areas, which measured 17.0 x 11.5 x 4.5 cm. Both the walls of the arenas and the smaller containers used for the experiments were painted with liquid Fluon. Fluon is an extremely slippery, non-stick polytetrafluoroethylene coating (Blades Biological Ltd.). Its usage on the walls of the containers prevents ants from being able to climb out and thus stops them from escaping whilst the food is changed. Figure 3.1 shows the ant arenas. The Fluon lining is visible within this Figure as a white border near the top of the tanks.

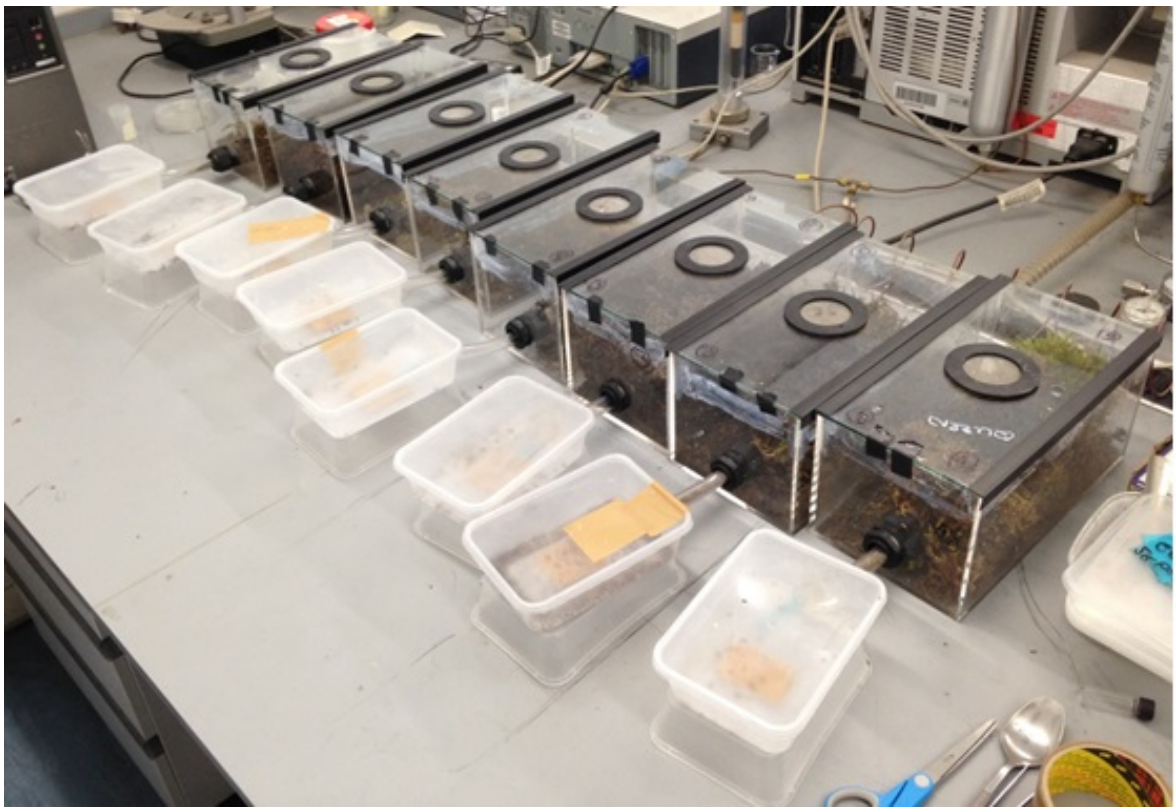


Figure 3.1: Typical ant arena setup, with attached foraging boxes.

Throughout the project the ants were fed regularly with a diet based on that of Bhatkar and Whitcomb [1]. This diet is widely used within ant research as it provides the ideal blend of sugars, protein, vitamins and minerals and is suitable for varied genera [2–6]. For the duration of this study however the published diet was modified slightly; a whole egg was replaced with powdered egg white to provide protein with minimal waste. Once made, this food has a gelatinous consistency, this means that it can be easily divided into controlled size portions and placed onto small trays for feeding.

3.2 Chemical Analysis

3.2.1 Extraction method

The various methods for the extraction of cuticular hydrocarbons have already been discussed in Section 2.2. For this study whole body extracts were used as they ensure the greatest amount of hydrocarbons are removed and are relatively quick and simple to perform. The study by Vander Meer suggested an extraction time of seven minutes was optimum, however there was little discernible difference up to a 15 minute extraction length [7]. Therefore for this project all whole body extracts utilised a 15 minute extraction time to minimise the risk of internal contamination, whilst ensuring all cuticular hydrocarbons were effectively removed.

Upon ceasing an experiment, prior to extraction, the experimental groups of ants were removed from the experimental containers and placed into empty Fluon lined boxes. These boxes were then placed in the freezer at -18 °C for 30 minutes to kill the ants. This ensured that the extraction process was as stress free as possible, as stress has been shown to cause the ants to regurgitate pharyngeal contents into the solvent (S Martin, pers. comm.) Once killed each ant was removed from the box and placed into a standard sprung vial insert contained within a glass GC vial. To this the minimum amount of *n*-hexane (Sigma Aldrich) was added so that the ant was fully immersed and the CHCs removed. After approximately 15 minutes the ant was removed and discarded. The remaining *n*-hexane was evaporated to dryness and 30 μL of *n*-hexane was added ready for injection in the GC-MS, see Figure 3.2 for a schematic of this extraction process.

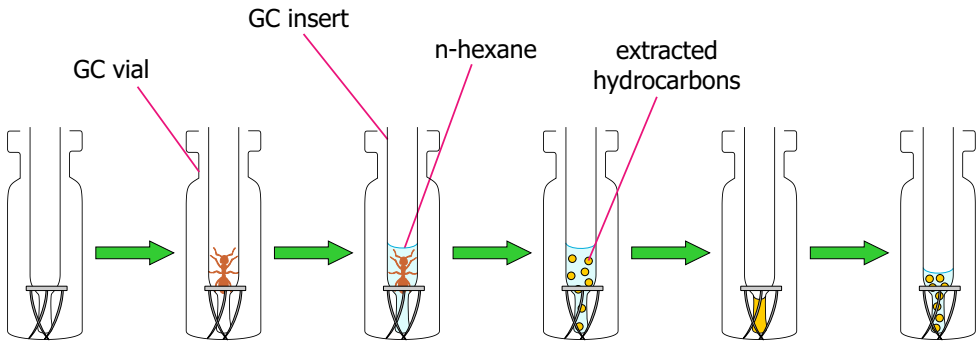


Figure 3.2: Standard extraction method used to remove cuticular hydrocarbons. The schematic shows how the evaporation and re-addition of solvent effectively concentrates the amount of CHC prior to analysis.

3.2.2 Instrumentation and analysis

Extracts were run on an Agilent 7890 gas chromatograph coupled to an Agilent 5975 MSD mass spectrometer (MS -70 eV, electron impact ionization) with a quadrupole mass analyser. The GC was equipped with a Vf-5ht UltiMetal column (length, 30 m; ID, 0.25 mm; film thickness, 0.1 μm; Varian Inc. USA) capable of withstanding higher temperatures than a standard column. The oven temperature programme was initially 70 °C and this was held for one minute. A temperature ramp of 40 °C min^{-1} to 200 °C was then performed followed by a slower temperature ramp of 4 °C min^{-1} to 250 °C. Finally there was a 25 °C min^{-1} temperature ramp finishing at 350 °C. This temperature was held for five minutes. All samples were injected in splitless mode with the injection port heated to 325 °C and the MS in scan mode. Helium was used as the carrier gas and the flow rate was 1.0 mL min^{-1}. All hydrocarbons were identified using diagnostic ions and Kovats indices [8]. All spectral analysis was performed using the Agilent ChemStation software.

3.3 Chemical Profiling

3.3.1 Identification of species

Throughout the duration of this project a variety of ant species were collected and used for experimentation. Whilst the majority of species collected appeared to be morphologically distinct and therefore easy to identify, many *Myrmica* species appear morphologically similar. Due to this any *Myrmica* colony fragments collected had to be identified prior to use. Figure 3.3 shows images of each of the species used during this project, the similarities between some of the species can be easily seen.

(a) Image of a *Formica lugubris* ant

(b) Image of a *Formica lemani* ant

(c) Image of a *Myrmica scabrinodis* ant

(d) Image of a *Myrmica sabuleti* ant

Figure 3.3: Images of the various ant species used within this project, all images were taken using a Canon 7D DSLR camera fitted with a 100 mm macro lens.

It has long been known that CHC profiles are species specific and hence can aid species identification [9–12]. Therefore the CHCs were used to confirm, and in some cases iden-

tify, the ant colonies prior to any experimental work. In order to identify the species of each colony fragment the procedure detailed in Section 3.2.1 was used to sample 10 ants from each fragment and from the resulting chromatograms and mass spectra, the main compounds were identified. From this the identity of the species could be determined or confirmed.

Figure 3.4 shows example chromatograms of the cuticular hydrocarbon profiles of the ant species used in this project. *Formica lugubris* is characterised by the large pentacosane peak and abundance of small quantities of methyl and dimethyl compounds eluting later in the chromatogram. The cuticular hydrocarbon profile of *Formica lemani* is fairly simple and is characterised by the large pentacosene and heptacosene peaks. The two *Myrmica* species all appear similar as they all contain large amounts of pentacosene. Based on Guillem et al. [10] the key difference between the two *Myrmica* species is the presence of 5-methylpentacosane in *M. sabuleti* and 3-methyltricosane in *M. scabrinodis*. 5 methyl-pentacosane is indicated by the dark grey oval in Figure 3.4. As these chromatograms show, despite the similar appearance of some species, all can be easily chemically identified. See Appendix A for full chromatograms and compound tables for each species listed here.

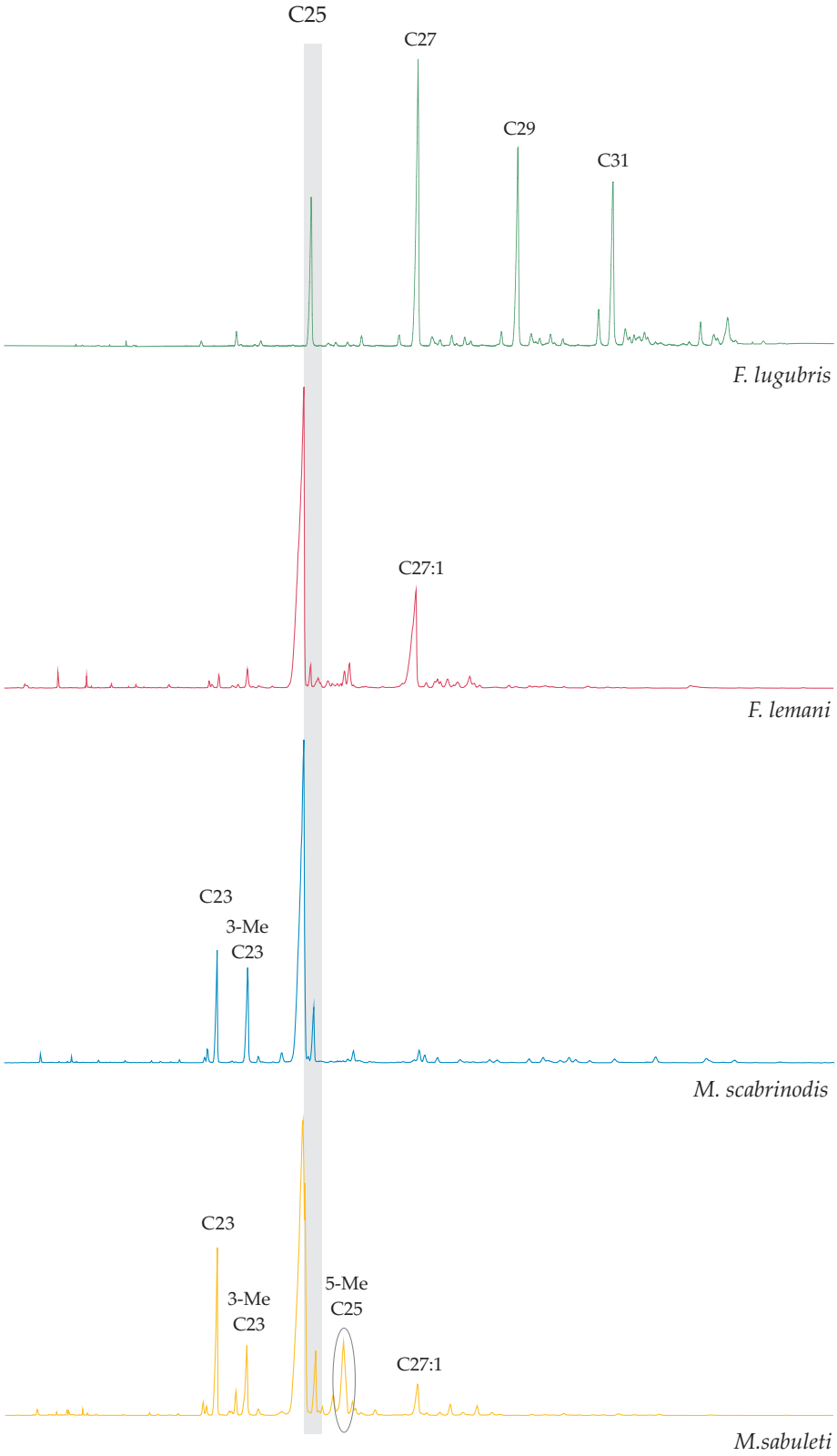


Figure 3.4: The CHC profiles of the main four British ant species used in this project, the grey bar indicates the position of pentacosane, whilst the grey oval indicates the difference between *M. scabrinodis* and *M. sabuleti*.

3.3.2 Identification of double bond position

Whilst extracting CHCs provides basic information about the compounds present on the cuticle it cannot identify the position of the carbon-carbon double bond within alkenes. One of the ways in which the position of these double bonds can be identified in alkenes is through dimethyl disulfide reactions. Therefore DMDS reactions were performed on each identified species; *Myrmica sabuleti*, *Myrmica scabrinodis*, *Formica lemani* and *Formica lugubris*. Due to their small size, and therefore low predicted amounts of CHCs, for both *Myrmica* species samples were pooled extracts of 10 ants. As *F. lemani* are generally larger, five ants per sample were combined together. For *F. lugubris* individual ants could be used due to their large size (∼10 mm). The CHCs were extracted according to the method described in Section 3.2.1, however this method varied slightly in that sprung vial inserts were not used, to facilitate the later DMDS reaction. Once extracts had been evaporated to dryness, 50 μL of *n*-hexane was added to dissolve the CHCs present. To this 100 μL of dimethyl disulfide and 50 μL of saturated iodine solution in diethyl ether was added, in a method adapted from Carlson et al. [13]. The samples were heated overnight at 60 °C and after, saturated sodium thiosulfate solution was added to decolourise the iodine. The organic layer was extracted three times into a second flat bottomed glass insert ready for analysis (50 μL of hexane added each time.) This was left to evaporate until approximately 50 μL of solvent remained. At this point each of the samples was injected according to the method described in Section 3.2.2.

An introduction to the analysis of DMDS spectra can be found in Section 2.4.4. As discussed this reaction involves adding two CH_3S groups across the double bond of an alkene. When fragmented by a mass spectrometer, characteristic fragments are produced as cleavage occurs at the carbon-carbon bond [13]. See Figure 2.13 in Chapter 2 for a schematic of this process.

As previously described the analysis of the characteristic fragments produced by this

reaction yields the position of the double bond. See Figure 3.5 for an example of a mass spectrum showing the presence of these characteristic DMDS fragments.

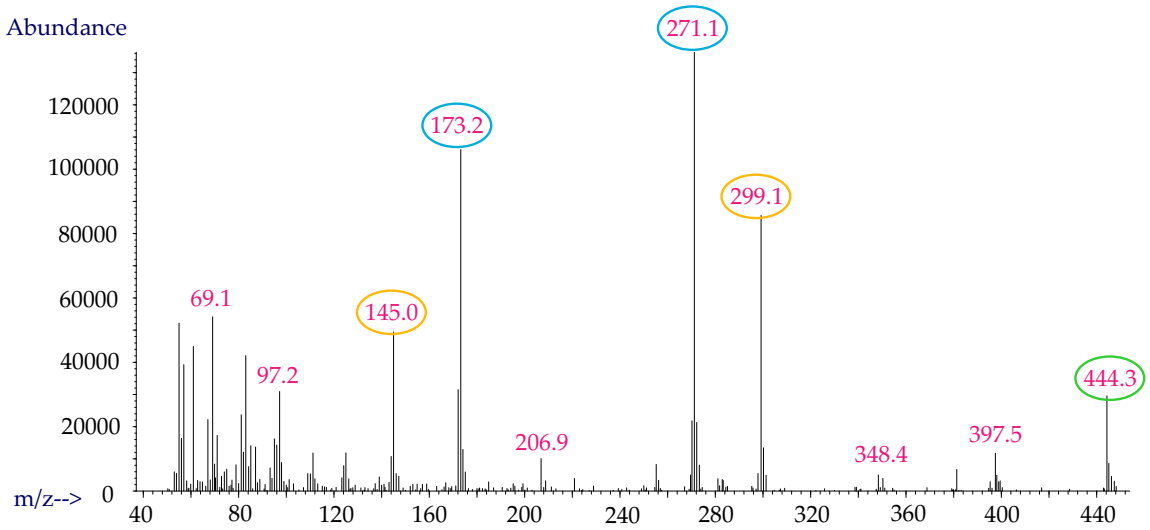


Figure 3.5: The resulting mass spectrum of pentacosene following a DMDS reaction. The yellow and blue circles indicate the pairs of characteristic fragments, which indicate the positional isomers present.

The first step in elucidating the compound present is to identify the mass corresponding to $[M^{+\bullet}]$ (the molecular weight). This mass can be used to identify the chain length present; by subtracting the mass of two SMe groups from the value of $[M^{+\bullet}]$ and dividing this total by 14 (the molecular weight of a CH_2 group) the chain length is revealed. In the example above the value of $[M^{+\bullet}]$ is m/z 444.3. From this 94 is subtracted (the mass of two SMe groups), this yields m/z 350. This value is divided by 14 to indicate a chain length of 25 carbons and thus the compound pentacosene. From the same spectrum the double bond position can be easily determined by looking at the characteristic pairs of ions. These pairs are indicated in blue and yellow in the example shown in Figure 3.5. The combined mass of each pair of ions totals the molecular weight ($[M^{+\bullet}]$) of the derivatised compound, which in the example shown in Figure 3.5 is m/z 444.3, indicated by a green oval. Once the pairs of ions are identified, a simple calculation can be used to determine the position of the double bond that each pair refers to. An

example calculation of that performed is shown in Calculation 3.1. This example refers to the blue pair of characteristic ions.

$$\frac{173 - 47}{14} = 9 \tag{3.1}$$

Calculation 3.1 shows that initially 47 is subtracted from the smaller mass value of the ion pair, in this case *m/z* 173, (47 corresponding to the mass of one SMe group). This is then divided by 14 and the resulting number indicates the position of the double bond. As determined from $[M^{+\bullet}]$, the chain length is C25, therefore in this example the blue ion pair indicates a C25:1 Z9 isomer and the yellow a C25:1 Z7 isomer. These isomers are chemically so similar that they co-elute, meaning that on the chromatogram they appear as one peak, that of C25:1, hence correct identification of the ion pairs from the mass spectrum is an essential part of the process. These ion pairs can often be very low in abundance, however this technique still remains an extremely sensitive one. In the same way the DMDS derivatised samples for all four species were analysed. It was found that different species contained different positional isomers and different amounts. Table 3.1 shows the identified positional isomers for each species.

Table 3.1: The identified positional isomers for the various alkenes for each of the main four species of studied ants. For example the table shows *Formica lemani* produces only Z9 positional isomers; C25:1 Z9 and C27:1 Z9. Note that none of the species indicated any Z8 or Z10 compounds.

Colony	Positional Isomers				
	7	9	11	12	13
Formica lugubris		C25:1			
Formica lemani		C25:1, C27:1			
Myrmica scabrinodis	C23:1, C25:1, C27:1, C29:1	C25:1, C27:1, C29:1, C31:1	C27:1		
Myrmica sabuleti		C25:1, C27:1	C25:1	C25:1	C27:1

Table 3.1 shows that some of the species are rich in different isomers, whilst some

contain relatively few. Although the identification of the double bond position was not necessary to identify which species were present, it was necessary to perform this step so that the results from subsequent substrate experiments could be correctly analysed.

3.4 Substrate Experiments

3.4.1 Method

For the majority of this project substrate experiments have been used to provide information towards the elucidation of possible biosynthetic routes. The nature of all of these experiments is similar, in that groups of ants are fed varying diets and the resulting CHC profiles analysed to see if changes occur to the CHC compounds produced. For all experiments plastic boxes were used, these measured 17.0 x 11.5 x 4.5 cm and were prepared by painting the sides with Fluon anti-stick coating (Blades Biological Ltd.) and in some cases the bottom with a thin layer of plaster of paris, to help ensure a moist environment. A thin layer of soil was also added. To each box between 20 and 30 ants of the selected species were added. Generally all experiments ran for 25-30 days; as each experiment varies slightly, full experimental detail will be provided at the start of each chapter.

For each substrate experiment performed, modified diets were prepared. This was done using the same standard diet as that referred to in Section 3.1. To this basic diet 1-3% weight by weight (w/w) (normally 2% w/w) of the chosen labelled substrate was added, resulting in the substrate diet. In later experiments a control was also included, this was the same basic diet however it was modified with the addition of the unlabelled version of the chosen substrate. All diets were cut into small cubes (approximately 0.5 cm^3). One cube was placed on to a small piece of plastic inside each box and the food was replaced every other day. All diets were stored in the freezer and defrosted prior to use each time.

At the end of each substrate experiment all living ants were chemically extracted to remove the CHCs present according to the method described in Section 3.2.1 and subjected to GC-MS analysis using the method outlined in Section 3.2.2.

3.4.2 Analysis of results

3.4.2.1 Raw data handling

The analysis of the results from the substrate experiments relies on comparison of the substrate values to the control values in order to determine any differences in the chemical profiles of the substrate group. In the majority of experiments separate controls were run alongside the substrate groups. These controls consisted of groups of ants which were fed on a control diet; this was the same diet as the substrate group but with an unlabelled version of the substrate added. However for some substrate experiments inclusion of this type of control was not possible due to the substrate used. In this case the control values were taken from ants which were fed a standard diet i.e. no substrate included, either labelled or unlabelled. Further details on this process will be provided in the relevant sections.

The chemical profiles produced by ants can be complex with many compounds present in various abundances. Therefore for each species compounds were selected which were present in the chemical profile in high abundance. This was necessary to ensure that the resulting ion counts for each compound from the mass spectrum would be high enough to be accurate and meaningful. These compounds, referred to as the key compounds, were also selected on the basis of their type to ensure that alkenes, alkanes, and where relevant, monomethyl alkanes could be studied. Figure 3.6 shows the chromatogram produced by *M. sabuleti* as an example. The key compounds for this species are indicated by grey bars. Although not always the highest abundance compounds, these are the highest abundance of each type of compound for this species. During the analysis of the results, only the key compounds for each species were studied. It should be noted however that depending on the substrate experiment, slightly different compounds were selected due to natural compound abundances. Full details on this will be provided in each results chapter.

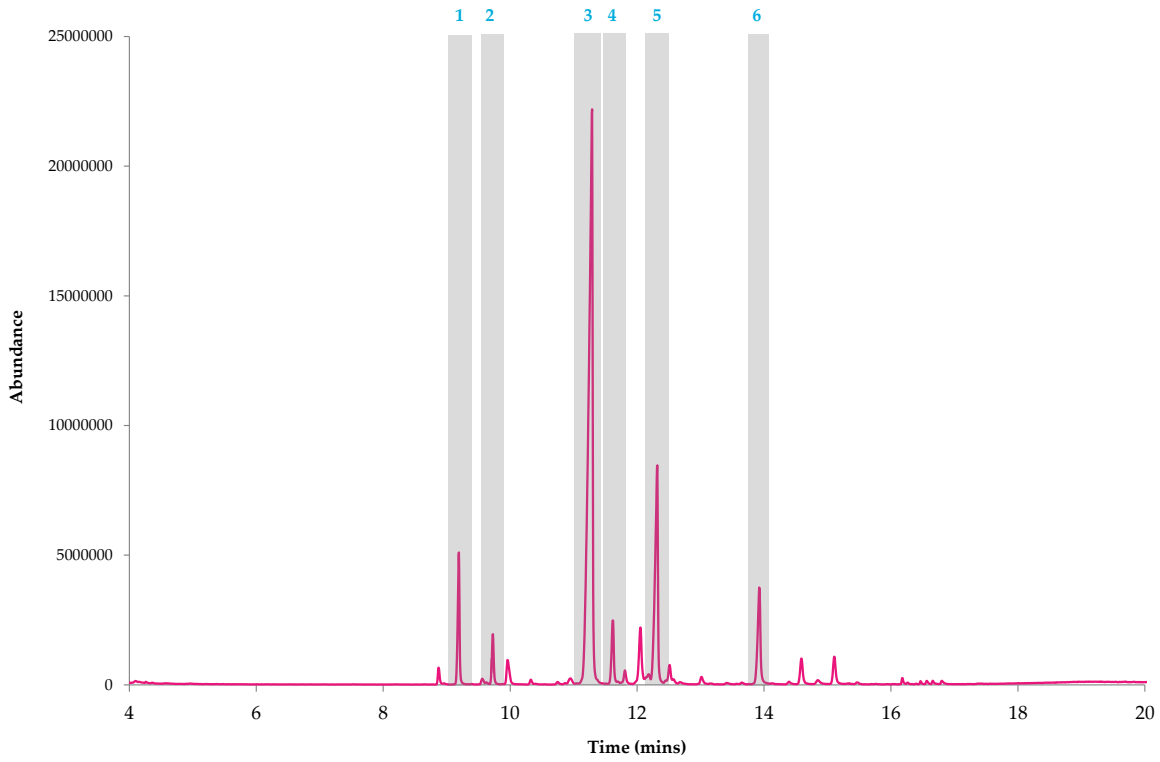


Figure 3.6: An example chromatogram of *M. sabuleti*. Each grey bar indicates a key compound of the species: 1) tricosane, 2) 5-methyltricosane, 3) pentacosene, 4) pentacosane, 5) 5-methylpentacosane and 6) heptacosene.

Once the key compounds for each species were established, the mass spectra of each compound was studied. Whilst the overall fragmentation pattern shown in the mass spectrum confirmed the compound identity, for the purposes of this project only the isomer peaks were studied. For an example analysis of a mass spectrum, in this case tricosane, see Figure 3.7. Note that for this example the molecular ion and the isomer peaks are shown in yellow and are indicated within the green box.

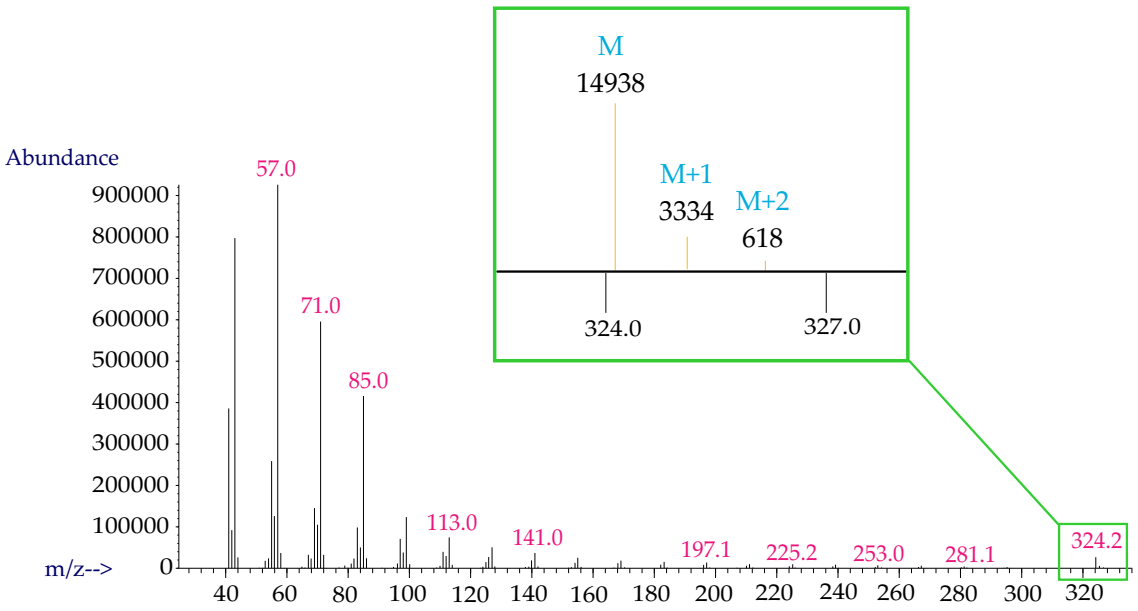


Figure 3.7: An example mass spectrum of tricosane. The area in the green box shows the molecular weight of the compound and the associated isotope peaks, with the ion counts of each also indicated in black.

For each key compound the intensity of the ion count, known as 'I', of the molecular ion peak represented here as 'M', was first identified and recorded. This provided a numerical value of the intensity of the molecular ion, referred to as $I^{(M)}$. Figure 3.8 shows an example data sheet. In this example, for the Control 1 sample the relative intensity (I) of the molecular ion (M) for tricosane (m/z 324) is 14938, indicated in pink.

		Control 1	Control 2	Control 3	Substrate 1	Substrate 2	Substrate 3
Ion count M	Tricosane	14938	36800	29496	4907	2308	8302
	Pentacosene	56920	98792	22728	8901	5470	18352
	Pentacosane	20768	25936	37272	6528	7013	8453
	5-methyl C25	66456	118792	47264	26960	18088	42584
	Heptacosene	13383	23216	6270	3012	1058	4776
Ion count M+1	Tricosane	3334	8366	6031	4191	1220	4961
	Pentacosene	15697	27048	5757	12226	3436	14676
	Pentacosane	4714	6253	8091	2162	2619	3921
	5-methyl C25	12726	25000	9358	22752	13740	31824
	Heptacosene	3942	7380	1710	2510	1291	3865
Ion count M+2	Tricosane	618	592	624	3386	789	4129
	Pentacosene	1912	3010	973	11386	3517	13902
	Pentacosane	763	438	1581	1808	1206	2765
	5-methyl C25	1702	3151	1646	16632	9342	21320
	Heptacosene	551	896	502	1403	1119	3493
Percentage of M+1	Tricosane	22.3	22.7	20.4	85.4	52.9	59.8
	Pentacosene	27.6	27.4	25.3	137.4	62.8	80.0
	Pentacosane	22.7	24.1	21.7	33.1	37.3	46.4
	5-methyl C25	19.1	21.0	19.8	84.4	76.0	74.7
	Heptacosene	29.5	31.8	27.3	83.3	122.0	80.9
Percentage of M+2	Tricosane	4.1	1.6	2.1	69.0	34.2	49.7
	Pentacosene	3.4	3.0	4.3	127.9	64.3	75.8
	5-methyl C25	3.7	1.7	4.2	27.7	17.2	32.7
	5-methyl C25	2.6	2.7	3.5	61.7	51.6	50.1
	Heptacosene	4.1	3.9	8.0	46.6	105.8	73.1

Percentage
0
0-20
20-40
40-60
60-80
80-100
100+

Figure 3.8: An extract from a substrate experiment data sheet, showing the ion count values for each compound and the resulting percentage abundance values. Note that for the purposes of these data sheets $I^{(M+1)}/I^{M}$ is represented by 'Percentage of M+1', and $I^{(M+2)}/I^{M}$ is represented by 'Percentage of M+2'.

Therefore in this example $I^{(M)}$ is 14938 counts. Once this value was recorded, the intensities for the isomer peaks were also identified. These isomer peaks are naturally present due to the abundance of natural isotopes of carbon and hydrogen which cause the compound to have a greater molecular weight. These isomer peaks are referred to as M+1, i.e. one mass unit (1 Da) heavier than the mass peak M, and M+2 i.e. two mass units (2 Da) heavier. In the same way the intensities of these peaks are tabulated and are referred to as $I^{(M+1)}$ and $I^{(M+2)}$. In the same example shown in Figure 3.8 the values of $I^{(M+1)}$ and $I^{(M+2)}$ are 3334 and 618 respectively. For ease of understanding these values are also highlighted in blue ($I^{(M+1)}$) and green ($I^{(M+2)}$). Collectively these isotope peaks are known as M+n, where n can equal 1, 2, or 3 etc.

Some of the compounds that were studied were methyl-branched compounds. Due to the energy involved in the fragmentation process, the molecular ion in the mass spectrum is rarely seen as it is unlikely that methyl-branched compounds survive the ionisation process intact. This is mainly due to the presence of the methyl branch. The bond energy associated with the carbon-carbon bond that forms the methyl-branch is much weaker than the carbon-carbon bonds that form the main chain and thus this bond is more readily broken. Whilst methyl-branched compounds rarely exhibit the molecular ion [$M^{+\bullet}$] the [$M^{+\bullet}$–15] peak is more readily observed. This corresponds to a fragment that has lost the methyl branch. However, although observable in most cases, this is still not a suitable peak within the mass spectra to base the isotope measurements on as it is generally not intense enough. Instead, for each methyl-branched compound a characteristic ion was selected from the mass spectrum that was in high abundance. The various intensities of M and M+n were then based on this fragment. Figure 3.9 shows the mass of the ion peak within the mass spectra that each measurement was based on, for each compound, across all ant species.

As discussed earlier this work utilised substrate experiments in which groups of ants were fed isotopically labelled substrates. If these substrates were incorporated into the final hydrocarbon via biosynthesis, then the isotopic label may also be included. If this label is included then the resulting hydrocarbon would be slightly heavier and the level of $I^{(M+X)}$ would be elevated. If there was no incorporation then the value of $I^{(M+X)}$ would be indicative of normal isotope values. However the intensity of the M and M+n peaks is related to the intensity of the compound itself and thus the concentration of the extraction, i.e. an extract which is highly concentrated will show greater abundance or intensity values than one which is weaker. The concentration of extracts can vary due to a number of reasons and cannot be controlled. This means that intensity values of M and M+n, as absolute ion counts, cannot be directly compared between samples. Therefore the value of $I^{(M+1)}$ is represented as a percentage of the value for $I^{(M)}$ and is referred to as the percentage abundance. Referring back to the example

	Formica lugubris	*Formica lemani*	*Myrmica sabuleti*	*Myrmica scabrinodis*	Ion used
Tricosene					322
Tricosane					324
5-methyl C23					**281**
3-methyl C23					**309**
Pentacosene					350
Pentacosane					352
11-, 13-, & 15-methyl C25					**196**
5-methyl C25					**309**
3-methyl C25					**337**
Heptacosene					378
Heptacosane					380
5-methyl C27					**337**
3-methyl C27					**365**
Nonacosene					406
Nonacosane					408
11-, 13-, & 15-methyl C29					**196**
Hentriacontene					434

Figure 3.9: A schematic showing a summary of the key compounds and the ions used during the various substrate experiments for each species. Note that the compound column on the left lists all the compounds studied across the various experiments, and does not represent any single experiment. Values in bold indicate where a ion fragment was used rather than the molecular ion

shown in Figure 3.7, the value for the percentage abundance of M+1 for would therefore be calculated as:

$I^{(M+1)}/I^{M}$ = (3334/14938) x 100 = 22.3%

In the same way, the value of M+2 would be calculated as:

$I^{(M+2)}/I^{M}$ = (618/14938) x 100= 4.1%

Using Excel the percentage values are calculated and added to the data sheet. Note that in this way the values calculated above are shown within the data sheet in Figure 3.8 under 'Control 1' in blue and green respectively.

In the same way, using an Excel spreadsheet, these calculations were performed for each compound for each sample, for both the substrate and control data sets, and tabulated. The percentage values were then compared and any statistical difference identified. In nature the natural abundance of ^{13}C is approximately 1.1%. This means that as well as the measured and calculated values from experimental data, the theoretical value for $I^{(M+1)}/I^{M}$ (control) can also be calculated using the formula below: [14]

$$\frac{I^{(M+1)}}{I^{M}} = \text{natural abundance of } ^{13}C \times \text{number of carbons in compound} \tag{3.2}$$

For example for pentacosane, which contains 25 carbons, the calculation would be: $I^{(M+1)}/I^{M}$ = 1.1 x 25 = 27.5%.

Due to the very low probability of having two ^{13}C atoms in the molecule the expected values for $I^{(M+2)}/I^{M}$ are much lower. However the value for $I^{(M+2)}/I^{M}$ can also be calculated using the more complicated formula below: [14]

$$\frac{I^{(M+2)}}{I^{M}} = 0.006 \times (\text{number of carbons in compound}^2) \tag{3.3}$$

Again for pentacosane, which contains 25 carbons, the calculation would be: $I^{(M+2)}/I^{M}$ = 0.006 x (25^2) = 3.75%.

Throughout this project experimentally obtained control values were used, however close correlation was seen between the experimentally derived data and the theoretical values.

3.4.2.2 Statistical analysis

Once all the studied values for $I^{(M+n)}/I^{M}$ had been calculated and tabulated, further statistical analysis was performed. As previously mentioned in Section 2.5.2, the data for these experiments was analysed using the Mann-Whitney U-test. This is a statis-

tical test that ranks the raw data in order to remove the large variances within each data set. In order to perform the Mann-Whitney U-test the percentage abundance values for each compound in each sample were first input into the statistical software package SPSS (IBM, US). Note that the calculations for the percentage abundance of M+1 and those of M+2 were performed separately. Figure 3.10 shows how the M+1 data would be input based on the example data sheet shown in Figure 3.8. The six columns refer to the five studied compounds and the final column refers to the group label. All substrate samples are marked as group two and all control samples as group one.

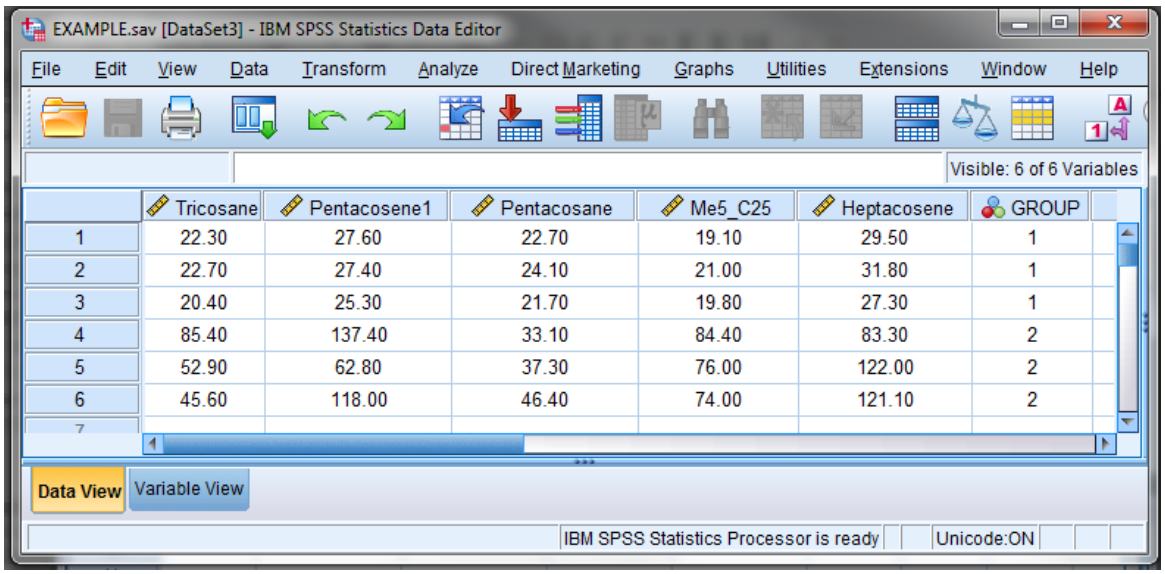


Figure 3.10: A screenshot taken from SPSS showing the data input. Note that the data shown is that from the example data shown in Figure 3.8, as such there are three control values and three substrate values.

Figure 3.11 shows an extract from the first part of the resulting SPSS output after the Mann-Whitney U-test has been performed. The extract shows the mean rank of each group for each of the studied compounds. The substrate group is represented by group 2 and the control is represented by group 1. The output file also shows the sum of the ranks for each compound, and N, the number of samples within each group.

	GROUP	N	Mean Rank	Sum of Ranks
Tricosane	1	3	2.00	6.00
	2	3	5.00	15.00
	Total	6		
Pentacosene	1	3	2.00	6.00
	2	3	5.00	15.00
	Total	6		
Pentacosane	1	3	2.00	6.00
	2	3	5.00	15.00
	Total	6		
5-methyl C25	1	3	2.00	6.00
	2	3	5.00	15.00
	Total	6		
Heptacosene	1	3	2.00	6.00
	2	3	5.00	15.00
	Total	6		

Figure 3.11: An extract from an SPSS output file showing the sum rank and mean rank values for each group, i.e. 1 and 2, (control and substrate respectively) for each compound.

Figure 3.12 shows the exact significance (1-tailed) p values for each compound. If these values are ≤ 0.050, then the null hypothesis, that the two populations show the same level of incorporation, is rejected. If these values are > 0.050, then there is no evidence to reject the null hypothesis, and therefore there is no statistical difference between the two groups [15, 16]. In the same way, for the example given in Figures 3.11 and 3.12, it can be seen that for all compounds there is statistical significance between the substrate group and the control group as p=0.050. Rather than present all the output data as shown in Figures 3.11 and 3.12, for each set of data only these probability values will be recorded to indicate significance.

Test Statistics[a]

	Tricosane	Pentacosene	Pentacosane	5-methyl C25
Mann-Whitney U	.000	.000	.000	.000
Wilcoxon W	6.000	6.000	6.000	6.000
Z	-1.964	-1.964	-1.964	-1.964
Asymp. Sig. (2-tailed)	.050	.050	.050	.050
Exact Sig. [2*(1-tailed Sig.)]	.100[b]	.100[b]	.100[b]	.100[b]
Exact Sig. (2-tailed)	.100	.100	.100	.100
Exact Sig. (1-tailed)	.050	.050	.050	.050
Point Probability	.050	.050	.050	.050

Test Statistics[a]

Figure 3.12: An extract from an SPSS output file showing the Mann-Whitney U-test statistics for each compound.

In addition to calculating p for each compound the data was visually represented using box and whisker plots. These show the median percentage abundance for each group and compound, as well as the range of the data, see Figure 3.13.

Whilst these box plots do not confirm any statistical significance they can be used to visually show any large differences between the data sets. If box plots show the two samples sets to be clearly different then there will almost certainly be a statistical difference between populations. However the Mann-Whitney U-test will clearly indicate significance even if the samples shown within the box plots appear to be similar. Each box within the box plot features a horizontal line across it, this indicates the position of the sample median known as Q2. The coloured portion of the box above and below Q2 represents the range of the data falling within the lower and upper quartiles known as Q1 and Q3. Finally the lines that extend from the box show the minimum and maximum values of the data (excluding outliers and extreme outliers). Outliers are defined as being one and a half times greater than the inter-quartile range (Q3-Q1) whilst extreme outliers are defined as being greater than three times the inter-quartile range.

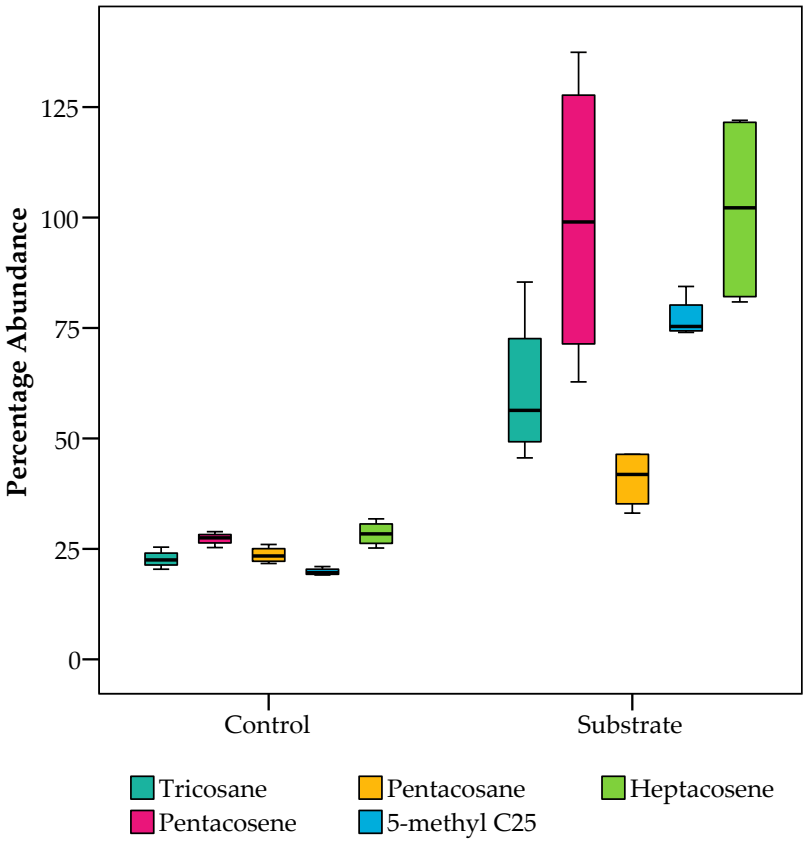


Figure 3.13: An example boxplot showing the median percentage abundance and range of each compound. Note that the data shown is that from the example shown in Figure 3.8.

In the same way, the previously described process was performed for all experimental data. As such, each set of results will consist of boxplots to visually represent the data and Mann-Whitney derived probability values to clearly indicate statistical significance. Where appropriate a representative mass spectrum will also be presented showing the ions counts for the molecular ion of the most abundant compound or other compound of interest, at all measured M+n levels for the substrate and control. This will allow for easy comparison between the mass spectra of the control and substrate data sets. The ion counts used for these representative mass spectra will be taken from the average ion counts across the data set so as to best represent the entirety of the data.

References

[1] Bhatkar A, and Whitcomb WH, 'Artificial diet for rearing various species of ants,' (1970), *Florida Entomol.*, 53(4), 229-232

[2] Martin SJ, Shemilt S, and Trontti K, 'Nest-mate recognition cues are not used during or influenced by mating in the ant *Formica exsecta*,' (2013), *Ethol. Ecol. Evol.*, 26, 40-48

[3] Chernenko A, Holman L, Helanterä H, and Sundström L, 'Cuticular chemistry of males and females in the ant *Formica fusca*,' (2012), *J. Chem. Ecol.*, 38(12), 1474-1482

[4] Khuong A, Lecheval V, Fournier R, Blanco S, Weitz S, Bezian J-J, and Gautrais J, 'How do ants make sense of gravity? A Boltzmann Walker analysis of *Lasius niger* trajectories on various inclines,' (2013), *PLoS One*, 8(10), 1-17

[5] El-Showk S, Van Zweden JS, D'Ettorre P, and Sundström L, 'Are you my mother? Kin recognition in the ant *Formica fusca*,' (2010), *J. Evol. Biol.*, 23(2), 397-406

[6] Nash D, Als TD, and Boomsma JJ, 'Survival and growth of parasitic *Maculinea alcon* caterpillars (Lepidoptera, Lycaenidae) in laboratory nests of three *Myrmica* ant species,' (2011), *Insectes Sociaux*, 58(3), 391-401

[7] Vander Meer R, Saliwanchik D, and Lavine B, 'Temporal changes in colony cuticular hydrocarbon patterns of *Solenopsis invicta*,' (1989), *J. Chem. Ecol.*, 15(7), 2115-2125

[8] Carlson DA, Bernier UR, and Sutton B, 'Elution patterns from capillary GC for methyl-branched alkanes,' (1998), *J. Chem. Ecol.*, 24(11), 1845-1865

[9] Seppä P, Helanterä H, Trontti K, Punttila P, Chernenko A, Martin SJ, and Sundström L, 'The many ways to delimit species: hairs, genes and surface chemistry,' (2011), *Myrmecol. News*, 15, 31-41

[10] Guillem RM, Drijfhout FP, and Martin SJ, 'Using chemo-taxonomy of host ants to help conserve the large blue butterfly,' (2012), *Biol. Conserv.*, 148(1), 39-43

[11] Urech R, Brown GW, Moore CJ, and Green PE, 'Cuticular hydrocarbons of the buffalo fly, *Haematobia exigua*, and chemotaxonomic differentiation from horn fly, *H. irritans*,' (2005), *J. Chem. Ecol.*, 31(10), 2451-2461

[12] Moore HE, Adam CD, and Drijfhout FP, 'Potential use of hydrocarbons for aging *Lucilia sericata* blowfly larvae to establish the postmortem interval,' (2012), *J. Forensic Sci.*, 58, 1-9

[13] Carlson DA, Roan CS, Yost RA, and Hector J, 'Dimethyl disulfide derivatives of long chain alkenes, alkadienes and alkatrienes for gas chromatography/mass spectrometry,' (1989), *Anal. Chem.*, 61(14), 1564-1571

[14] Sparkman O, (2010) *Separation Science 'MS Solutions'* at http://www2.spectroscopy-solutions.org/docs/Bespoke/Editions/MSSolutions/MSSol5EU.pdf (Accessed July 2017)

[15] Miller JN, and Miller JC, *Statistics and Chemometrics for Analytical Chemistry, 6th Edition*, (Prentice Hall, 2010)

[16] Ennos R, *Statistical and Data Handling Skills in Biology, 3rd Edition*, (Pearson, 2012)

Chapter 4

Labelled substrate feeding experiments: acetates and propionates

4.1 Introduction

The first of the substrate experiments were preliminary experiments in order to test the experimental methods, and to ensure that subsequent work would be able to follow the same established procedure. Within the literature there are many studies which have utilised labelled salts in order to gain some understanding of the biosynthetic processes of various insect species [1–5]. These salts are typically acetates or propionates and are labelled with either carbon-13 [2, 3], carbon-14 [1, 4] or deuterium atoms [5]. As described in Chapter 1, acetates are simple molecules used to lengthen hydrocarbons by two carbon units, for each molecule of acetate incorporated [6]. As such, they are used during the biosynthesis of a variety of compounds including fatty acids, alkenes and alkanes. Whilst propionates are used to biosynthesise even chain length *n*-alkanes (not often found in insect species), they are more commonly associated with the biosynthesis of branched hydrocarbons and are therefore first converted to methylmalonate. As such they are thought to only introduce the methyl-branches of these hydrocarbons, unless inserted at the terminus of the chain, in which case an even chain length *n*-alkane will result.

The work presented within this chapter consists of several experiments, divided into

three blocks. The first block consists of two consecutive experiments which tested the incorporation of two acetate compounds; sodium [1-^{13}C]acetate and sodium [2-^{13}C]acetate. Each of these compounds contained a single ^{13}C label which was positioned at a different point in the carbon chain. As these were the first experiments to be performed, three British ant species were tested; *Formica lugubris, F. lemani* and *M. sabuleti*. Using three different species allowed the suitability of each to be established and evaluated. Based on these initial findings the species used for further experimentation were selected. The second block consisted of an experiment which tested concurrently the incorporation of two different labelled propionate compounds; sodium [1-^{13}C]propionate and propionic-2,2-d_2 acid-d. These experiments were both preliminary experiments, mainly performed to gain very basic information about the biosynthesis of cuticular hydrocarbons in ants. However, they also provided a straightforward way to test the experimental design, and analytical and statistical methods.

The results of these preliminary experiments provided insight towards the direction of future research. Therefore the third block of experimentation focussed around expansion of previous work. As such two further experiments were performed using the substrate propionic-2,2-d_2 acid-d and two *Myrmica* species; *M. sabuleti* (as an expansion of the preliminary experiment mentioned earlier) and *M. scabrinodis*, a species new to this study.

The benefits of starting with simple salt substrates were numerous. Firstly there was plenty of research available utilising these types of substrates [1–5]. The results of these studies and the existing knowledge of biosynthetic pathways also gave a fair level of confidence that incorporation would be seen and thus this experiment provided an efficient way of testing the experimental design. Another benefit to using these types of substrates was that there is a relatively large variety of [1-^{13}C] and [2-^{13}C] labelled salts readily available from commercial suppliers, making their use relatively cheap and easy.

Aims & Objectives

This chapter will present the results from the first of the substrate experiments. These experiments focussed on the use of very simple labelled compounds: acetates and propionates, and the incorporation of these compounds into the biosynthesised hydrocarbons of four different British ant species. The species used within this chapter were; *Formica lugubris*, *Formica lemani*, *Myrmica sabuleti* and *Myrmica scabrinodis*. The aim of these experiments was to test the experimental methods as well as forming part of the overall results for each species.

4.2 Experimental

4.2.1 Preliminary acetate experiment

Experimental work using labelled acetate substrates was split into two, consecutively run, individual experiments. The first of the two experiments used sodium [1-^{13}C]acetate, whilst the second used sodium [2-^{13}C]acetate. See Figure 4.1 for the structures of these substrates. Labelled sodium acetate has been widely used in previous experimental work, by various researchers, for both ant [4, 7] and non-ant species [2, 3]. The experimental method for each of these experiments was kept consistent.

Sodium [1-^{13}C]acetate Sodium [2-^{13}C]acetate

Figure 4.1: Structure of the substrates used for the initial acetate experiment.

For each experiment six plastic boxes measuring 17.0 x 11.5 x 4.5 cm were prepared by painting the sides with Fluon anti-stick coating (Blades Biological Ltd.) A small amount of soil was also added to each box. For each species a control box and a substrate box was set up. To each box 30 worker ants of the three different species were added. The species were; *F. lugubris*, *F. lemani* and *M. sabuleti*. See Figure 4.2.

For each experiment modified diets were prepared using the standard diet as a basis, (see Section 3.1). To 50 g of this diet, 1 g of sodium acetate trihydrate (2% w/w) was added. This diet served as a control. Each substrate diet was made by adding either 1 g of sodium [1-^{13}C]acetate or sodium [2-^{13}C]acetate to 50 g of standard diet to give a resulting concentration of 2% w/w. All of the above chemical compounds were purchased from Sigma Aldrich (99% purity). All diets were cut into small cubes (approximately 0.5 cm^3). One cube was placed on to a small piece of plastic inside

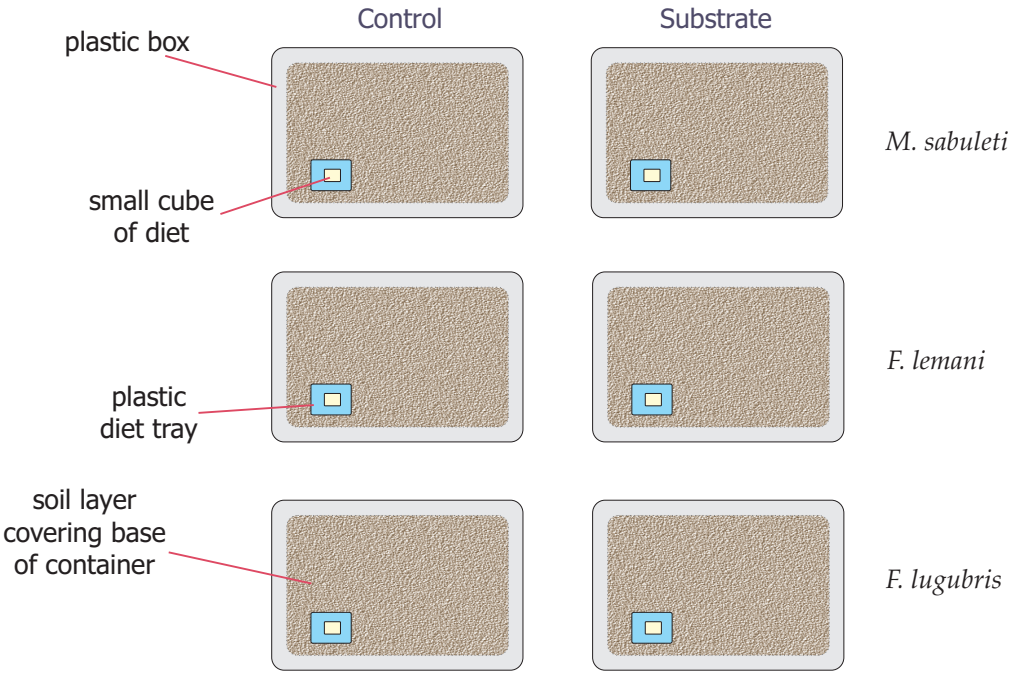


Figure 4.2: A birds eye view of the experimental setup used for the substrate experiments.

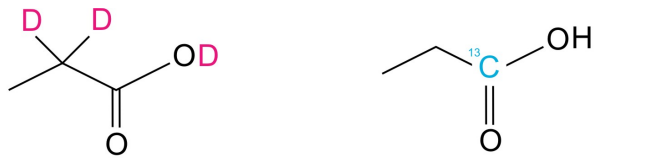
each box and the food was replaced every other day. All diets were stored in the freezer and defrosted prior to use each time. The experiment ran for 30 days.

After 30 days, ten ants from each of the six boxes were extracted and analysed, according to the methods described in Sections 3.2.1 and 3.2.2. For each species the chemical profile was studied and five key compounds were selected for analysis purposes. The compounds that were chosen were the most abundant for each species; they also represented the different compound types present. The most abundant compounds were chosen because the greater the amount of a particular compound present in the chemical profile, the higher the associated ion count from the MS detector, and thus the greater the accuracy of the measurement. Due to the abundances of the various compounds being species specific the compounds chosen varied, however the identity of these compounds can be seen within the presentation of the data. Note that for 3- and 5-methylpentacosane, the main fragment ions of m/z 337 and m/z 309 respectively

were used; this is because methyl-branched compounds rarely exhibit the molecular ion in the mass spectrum due to fragmentation.

4.2.2 Preliminary propionate experiment

The second block of preliminary experimentation focussed on the use of propionate compounds. In the same way as that previously mentioned, diets were made up using two labelled propionate substrates; sodium [1-^{13}C]propionate and propionic-2,2-d_2 acid-d. These two substrates were run concurrently with a control of unlabelled propionic acid and all substrate concentrations were 2% w/w. All propionate substrates, including the control, were purchased from Sigma Aldrich. Again the same three species were tested with one box per compound per species used, therefore in total there were nine boxes for this experiment.



Propionic-2,2-d_2 acid-d Sodium [1-^{13}C]propionate

Figure 4.3: The substrates used for the propionate experiment.

All other experimental details are as previously described in Section 4.2.1. As the feeding period progressed it was noted that there were very few live *F. lugubris* ants remaining. For this reason this experiment was stopped after 21 days, slightly earlier than previously. All extraction and analyses were carried out as described in Sections 3.2.1 and 3.2.2.

In contrast to the preliminary acetate experiments there were two changes made to the analysis of the results. The first was that the number of compounds studied for each data set was increased so as to include at least two alkene, alkane and methyl-

branched compounds per species. In addition, as many individuals as possible were sampled, which in many cases was far greater than the ten used previously. It was hoped that this change would increase the statistical strength of the data set.

4.2.3 Propionate experiment

The results of the two preliminary experiments described above were used to provide direction for the third block of experiments. For this it was decided to focus on two *Myrmica* species; *Myrmica sabuleti* and *Myrmica scabrinodis*. Several changes were also made to the experimental design to make it more rigorous. Firstly several repeats were performed; therefore instead of having one box per substrate per species, this experiment had five boxes per substrate per species. It was hoped that this would provide information on the variability of the incorporation within the experiment. In addition a further control substrate was added; as previously described this experiment focussed on propionic-2,2-d_2 acid-d, therefore propionic acid was used as onecontrol. However sodium [1-^{13}C]acetate was also included as an additional control. From the preliminary experimentation it is known that this substrate provides a reliable positive result. It would therefore provide an indication that the ants were successfully feeding. Therefore any negative results would be attributed to a lack of incorporation, rather than the experiment not running for long enough or a lack of feeding. This experiment therefore consisted of 30 individual boxes (two species, three substrates, five repeats per substrate). Each box contained approximately 30 ants.

4.3 Results & Discussion

4.3.1 Preliminary acetate experiment

As previously mentioned, due to the large variances within the raw data, and the fact that the data was not normally distributed, Mann-Whitney U-tests were performed on each data set to establish its significance compared to the relevant control. To visually show the data, box plots were also created, each showing the median and range of the data. However, whilst a visual representation of the data, these box plots do not confirm significance.

For each species the data will be presented for the two substrates; sodium [1-^{13}C]acetate and sodium [2-^{13}C]acetate. Please note that the results for $I^{(M+1)}$ and $I^{(M+2)}$ will be presented side by side. All raw data for this chapter can be found in Appendix B.

4.3.1.1 *Formica lugubris*

Upon completing the experiment for *F. lugubris* and the substrate sodium [1-^{13}C]acetate, it became clear that there were very few ants remaining in the control box. Although the experiment started with 30 *F. lugubris* ants in the control box and 30 in the substrate box, at the end of the experiment only two live control ants remained. Therefore with only n=2 for the control data it was not possible to analyse the control data for this experiment. However for the substrate box it was possible to analyse the data as ten samples were collected from this box. Therefore in order to analyse these results the control data from the sodium [2-^{13}C]acetate experiment will be compared against the substrate data for the sodium [1-^{13}C]acetate experiment. Although the two sets of data were collected from two different experiments the control values should show little change between experiments as the percentage values are dependant on very consistent natural abundances of ^{13}Carbon. The consistency of these values can also be compared against calculated values. Please see the data in Figure 4.4.

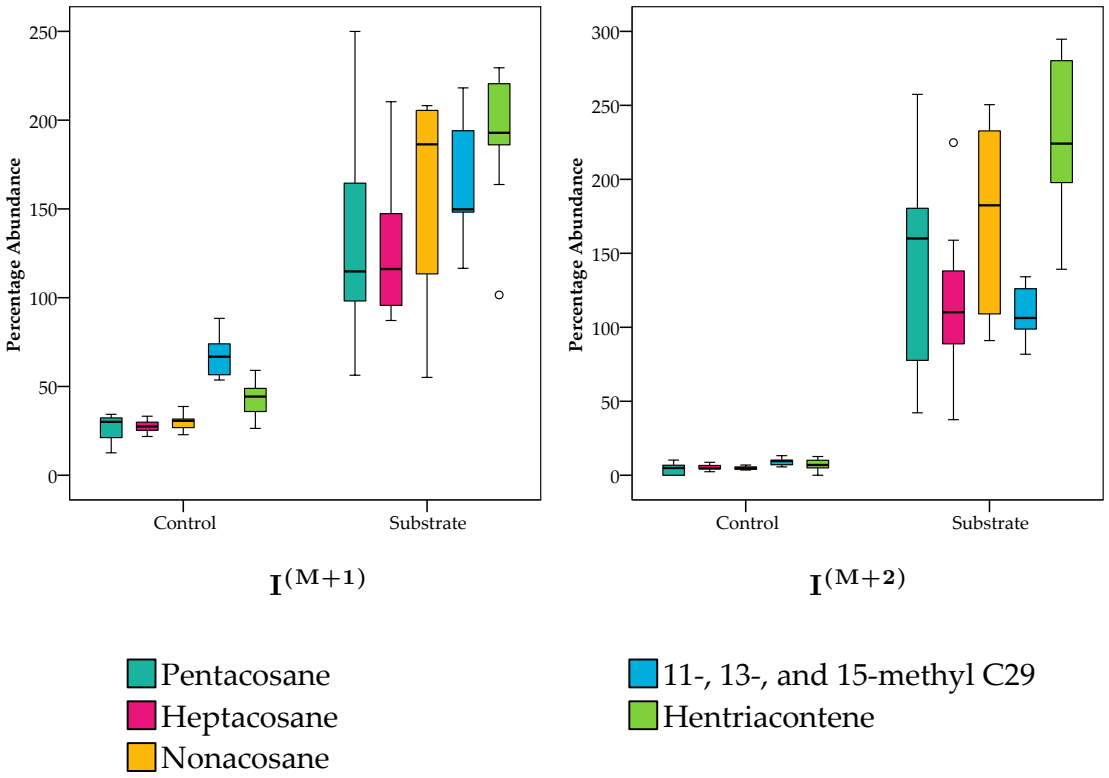


Figure 4.4: Boxplots showing the data for *Formica lugubris* and the substrate sodium [1-^{13}C]acetate; $I^{(M+1)}$ on the left, and $I^{(M+2)}$ on the right.

The boxplot data for *Formica lugubris* for the first substrate, sodium [1-^{13}C]acetate, for $I^{(M+1)}$ and $I^{(M+2)}$, shows that there is a clear difference in the two data sets. Whilst this data is comparing the substrate values and the control values from two different experiments it can be clearly seen that the two data sets are very highly likely to be statistically significant. The control data shows figures that are expected using Equation 3.2; the standard equation for calculating theoretical values for $I^{(M+1)}/I^{M}$. The boxplots indicate good consistency of these control values as evidenced by the small size of the boxes. Whilst this data comparison is not ideal, it does mean that the substrate data obtained from this experiment can be analysed and considered. The substrate data clearly shows that the intensity of the isotope peaks for these compounds is much greater than those for the control, with many instances of the intensity of the M+1 and M+2 peaks being far greater than that of the M peak, i.e percentage abundances greater than 100%. See Appendix B.1

The differences in the mass spectra between the two groups can be clearly shown by using a comparative mass spectra schematic shown in Figure 4.5. This example takes the average ion counts for the compound heptacosane (the most abundant) and compares them between the two data sets. Note that for this Figure the control data has been taken from the sodium [2-^{13}C]acetate experiment for consistency to the box plots above. Figure 4.5 shows that there is a clear difference between the mass spectra of the two groups; the appeance of the isotope peaks for the control is fairly typical, showing a steady downwards trend indicating that as the M+n level increases the number of counts decreases. This is compared to the average substrate data which shows that some istope levels were higher than the molecular ion, shown here as [M^+].

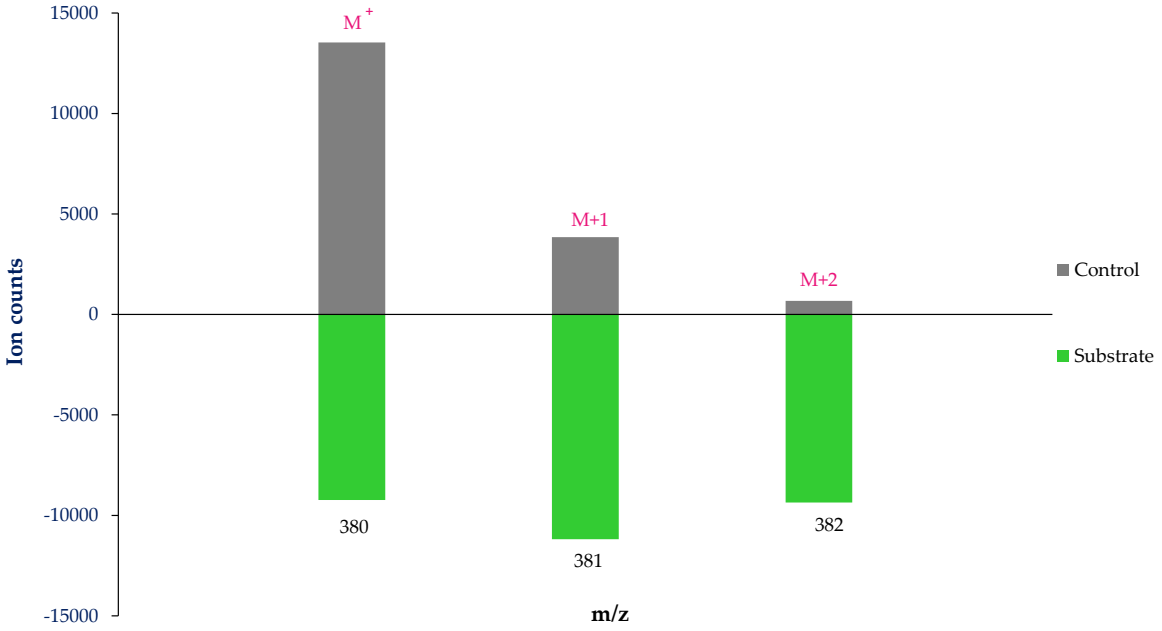


Figure 4.5: Extracted mass spectrum for *Formica lugubris* and the sodium [1-^{13}C]acetate experiment showing the average comparative ion counts for control and substrate data sets for the compound heptacosane.

The data for sodium [2-^{13}C]acetate was generally much better. At the end of this experiment there were far more ants remaining and therefore the data presented in Figure 4.6 is all from the same experiment and therefore directly comparable. Again clear differences in the intensities of the isotope peaks can be seen, see Figure 4.7 for

the comparative mass spectra. Figure 4.7 shows that for this substrate there was clear incorporation seen within the raw mass spectra, with elevated ion counts for the measured isotopes compared to the control group.

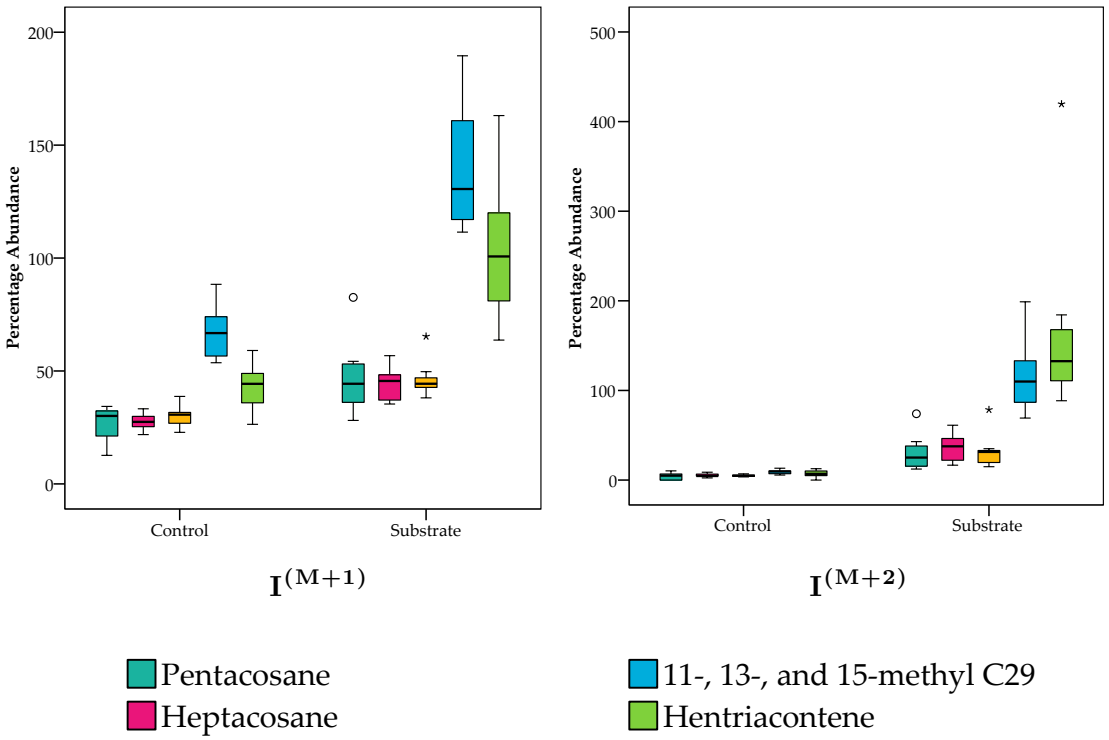


Figure 4.6: Boxplots showing the data for *Formica lugubris* and the substrate sodium [2-^{13}C]acetate; $I^{(M+1)}$ on the left, and $I^{(M+2)}$ on the right.

The boxes shown in the box plots for the substrate data are generally smaller for this experiment compared to that presented previously. This indicates that there is more consistency in the measured values across the data samples.

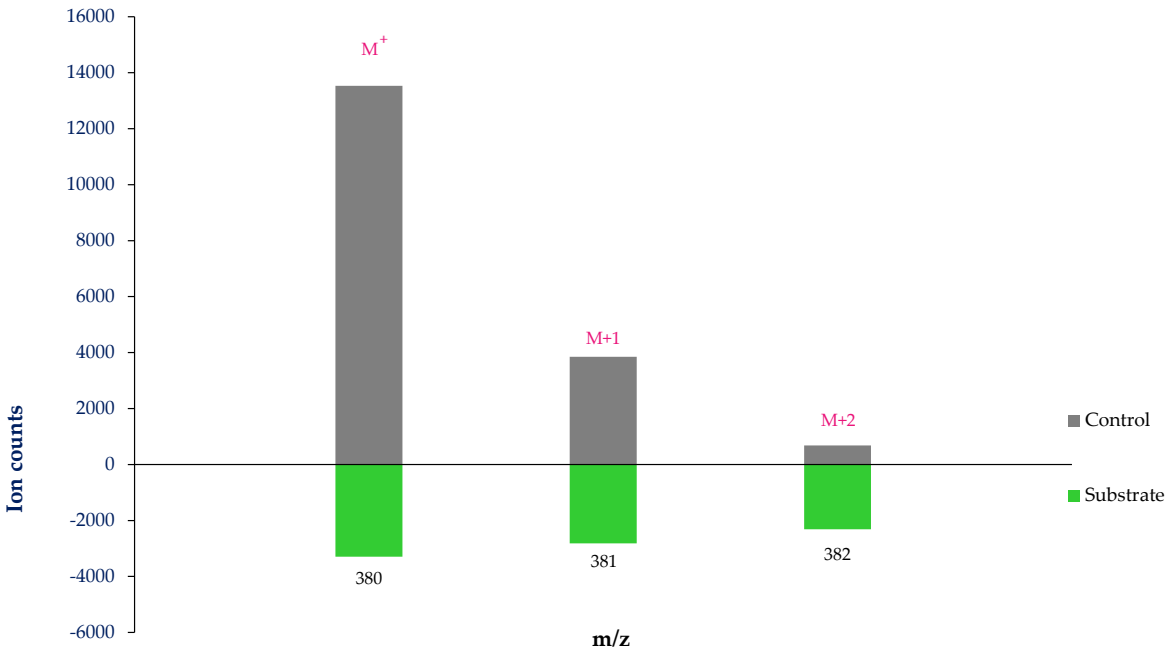


Figure 4.7: Extracted mass spectrum for *Formica lugubris* and the sodium [2-^{13}C]acetate experiment showing the average comparative ion counts for control and substrate data sets for the compound heptacosane.

In order to determine the statistical significance for the two substrates, Mann-Whitney U-tests were performed as previously described in Section 3.4.2.2. Table 4.1 shows the results of this test.

Table 4.1: The probability values from the calculated Mann-Whitney U-tests for *Formica lugubris*, for both substrates, and both isotope peaks.

Compound	p value			
	Sodium [1-^{13}C]acetate		Sodium [2-^{13}C]acetate	
	$I^{(M+1)}$	$I^{(M+2)}$	$I^{(M+1)}$	$I^{(M+2)}$
Pentacosane	0.000	0.000	0.002	0.000
Heptacosane	0.000	0.000	0.000	0.000
Nonacosane	0.000	0.000	0.000	0.000
11-, 13-, and 15-methyl C29	0.000	0.000	0.000	0.000
Hentriacontene	0.000	0.000	0.000	0.000

As confirmed by the Mann-Whitney U-test data shown in Table 4.1 statistical sig-

nificance was seen for all compounds, for both substrates and both isotope peaks. Additionally the table shows that the probability values are all ≤ 0.02. This is far lower than the critical probability value of ≤ 0.05. The result of this analysis therefore shows that *Formica lugubris* are able to use sodium acetate during the biosynthesis of hydrocarbons. The data indicates that there is strong incorporation for both substrates. These substrates differ only in the position of the labelled atom, therefore this data also shows that the carbon in both the first and second position of an acetate molecule can be incorporated into the final biosynthesised hydrocarbons for alkenes, alkanes, and methyl-branched hydrocarbons. This information can help to support previously established biosynthetic pathways and indicate that these routes are also applicable for this particular species of ants. A full discussion of these results will be provided at the end of the chapter.

4.3.1.2 *Formica lemani*

The same two substrates were used with the species *Formica lemani*. Unlike the data for *F. lugubris* this data was much better; sufficient numbers of both control and substrates samples were collected for each substrate (n=10). Figures 4.8 and 4.9 show the boxplots for the two substrate experiments for this species.

As shown in Figure 4.8, there are large differences in the median for each compound between the control and substrate data for both $I^{(M+1)}$ and $I^{(M+2)}$. Although only an indicator, large differences in the median values suggest that these sets may be significantly different from each other because they indicate that the percentage abundance was much higher for one of the groups, in this case the substrate group compared to the control group. The range of the data also varies between compounds, with some compounds having a small range and others, such as heptacosane, having a much larger spread of data for $I^{(M+1)}$ and $I^{(M+2)}$.

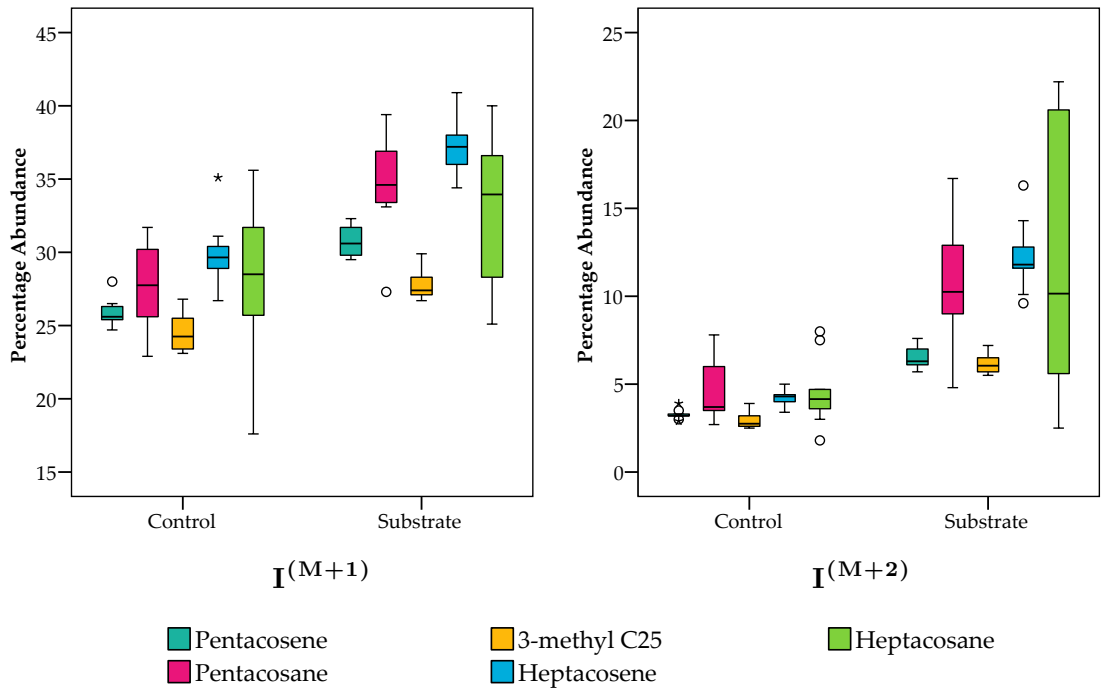


Figure 4.8: Boxplots showing the data for *Formica lemani* for sodium [1-^{13}C]acetate; $I^{(M+1)}$ on the left, and $I^{(M+2)}$ on the right.

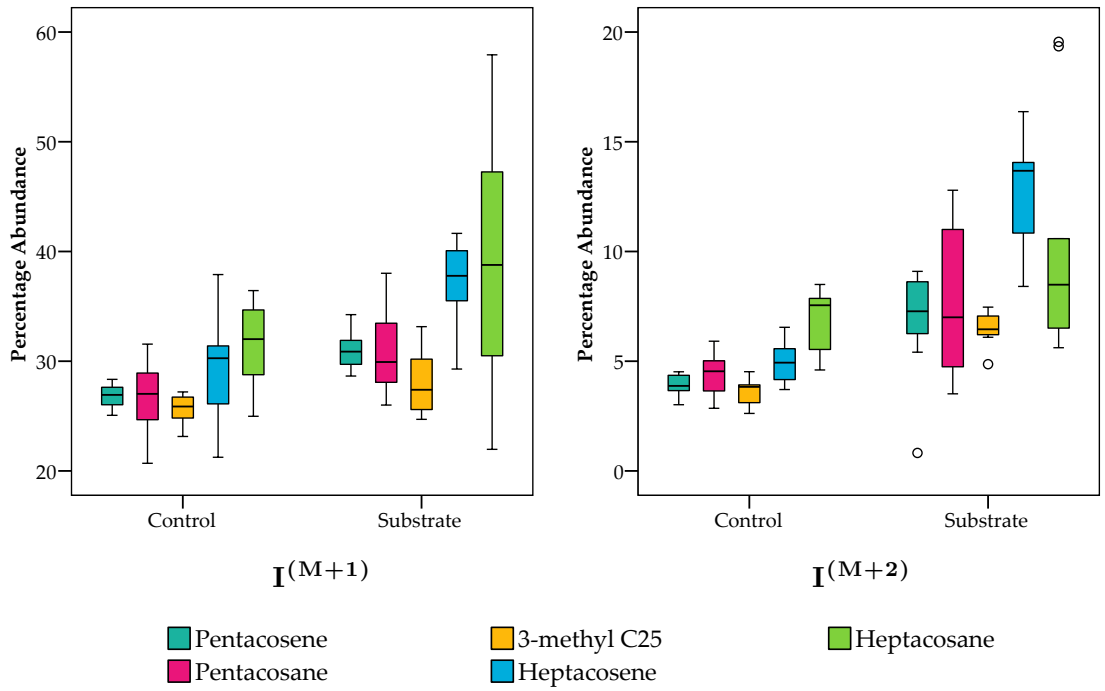


Figure 4.9: Boxplots showing the data for *Formica lemani* and the substrate sodium [2-^{13}C]acetate; $I^{(M+1)}$ on the left, and $I^{(M+2)}$ on the right.

As can be seen from Figure 4.9, again there are large differences in the median for each compound between the control and sodium [2-^{13}C]acetate substrate data for both

$I^{(M+1)}$ and $I^{(M+2)}$. The range of the data also varies between compounds, however generally the data for $I^{(M+2)}$ shows a much lower range of values than that for $I^{(M+1)}$. Overall for both substrates the difference between the control and sample populations is not as clear as that observed for *Formica lugubris*. The raw mass spectral data also did not show any clear difference in appearance between the two groups for either substrate and therefore mass spectra extracts will not be shown here. Mann-Whitney U-tests were performed to establish whether there was any difference in the percentage abundances of the isotope peaks for the two substrate and groups. Please see Table 4.2 for a summary of the Mann-Whitney U-tests.

Table 4.2: The probability values from the calculated Mann-Whitney U-tests for *Formica lemani*, for both substrates, and both isotope peaks. Note that any p values less than 0.050, and therefore significant, are underlined for ease of reference.

Compound	p value			
	Sodium [1-^{13}C]acetate		Sodium [2-^{13}C]acetate	
	$I^{(M+1)}$	$I^{(M+2)}$	$I^{(M+1)}$	$I^{(M+2)}$
Pentacosene	<u>0.000</u>	<u>0.000</u>	<u>0.000</u>	<u>0.001</u>
Pentacosane	<u>0.000</u>	<u>0.000</u>	<u>0.015</u>	<u>0.007</u>
3-methyl C25	<u>0.000</u>	<u>0.000</u>	<u>0.014</u>	<u>0.000</u>
Heptacosene	<u>0.000</u>	<u>0.000</u>	<u>0.001</u>	<u>0.000</u>
Heptacosane	0.074	<u>0.017</u>	0.099	<u>0.030</u>

When comparing the results between sodium [1-^{13}C]acetate and sodium [2-^{13}C]acetate, it can be seen that there is a similar trend between substrates, in that all compounds show incorporation with the exception of heptacosane, but only for $I^{(M+1)}$. These results therefore show that like *F. lugubris*, *F. lemani* also utilise sodium acetate molecules during the biosynthesis of alkane, alkene, and methyl-branched hydrocarbons. However the results show that the incorporation is not as great for this species. Possible reasons for these differences will be discussed at the end of this section.

4.3.1.3 *Myrmica sabuleti*

For this part of the experiment the number of samples was ten. The following figures show the boxplots associated with both of the tested substrates for the third study species; *Myrmica sabuleti*.

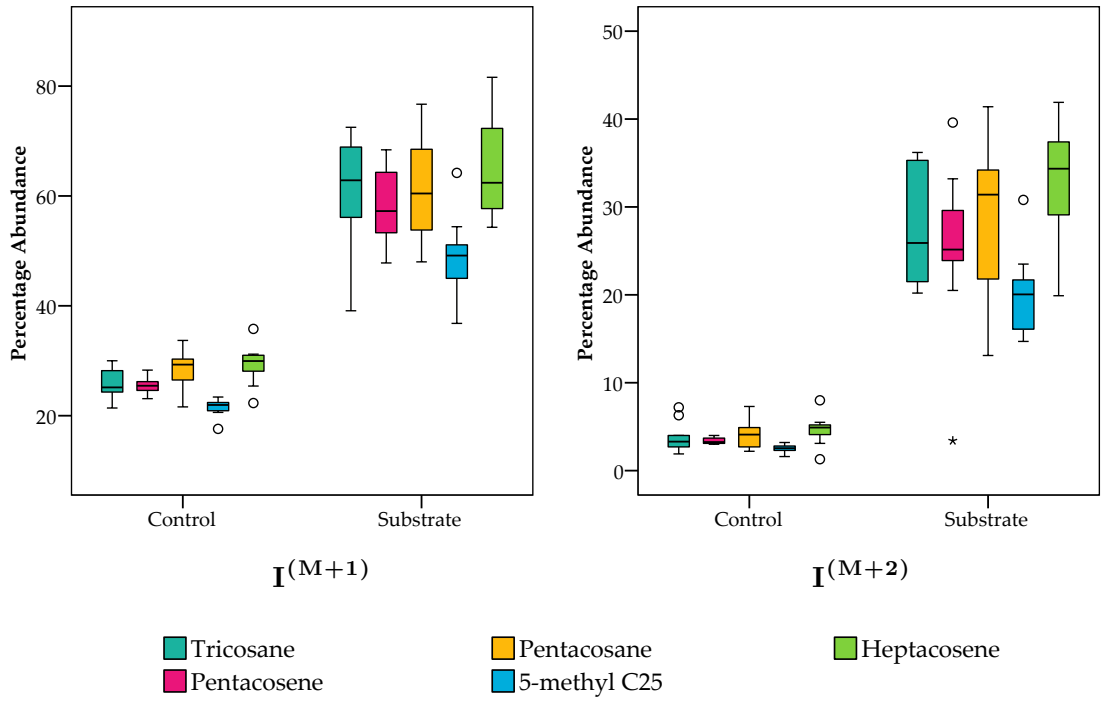


Figure 4.10: Boxplots showing the data for *Myrmica sabuleti* and the substrate sodium [1-^{13}C]acetate; $I^{(M+1)}$ on the left, and $I^{(M+2)}$ on the right.

The results for this set of data show that for this species there is far greater difference in the spread of the data between the control and the substrate with smaller boxes indicating a smaller range of data for the control, compared to the substrate. In addition there is a very clear difference in the medians for both sets of data. This indicates that the percentage abundance was much higher for the substrate group compared to the control group. As previously discussed typical theoretical percentage abundance values for $I^{(M+1)}$ would be around 25-30%, depending on the compound. However the raw data for this species indicated average percentage abundance values of 50-70%, see Appendix B.9.

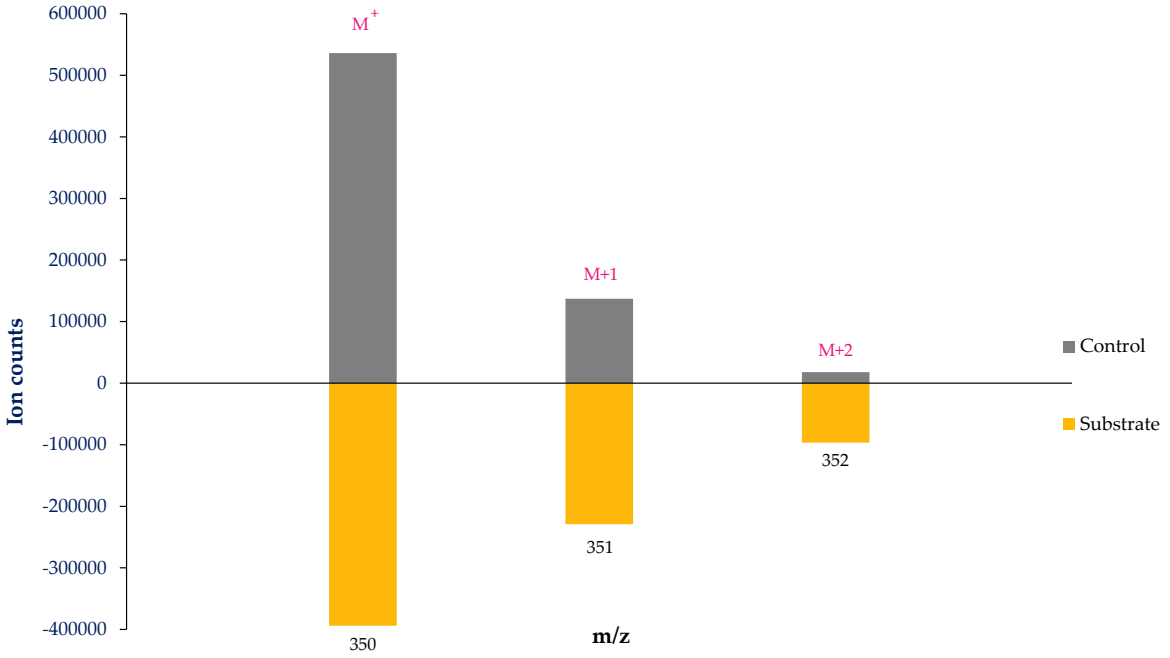


Figure 4.11: Extracted mass spectrum for *Myrmica sabuleti* and the sodium [1-^{13}C]acetate experiment showing the average comparative ion counts for control and substrate data sets for the compound pentacosene.

Figure 4.11 shows the comparative mass spectra for sodium [1-^{13}C]acetate, and the compound pentacosene. As this Figure shows there is a small difference in the appearance of the mass spectra between the substrate and control group; the average ion count for M+2 is unusually high for the substrate group compared to the control.

For the results of the sodium [2-^{13}C]acetate experiment, please see Figure 4.12.

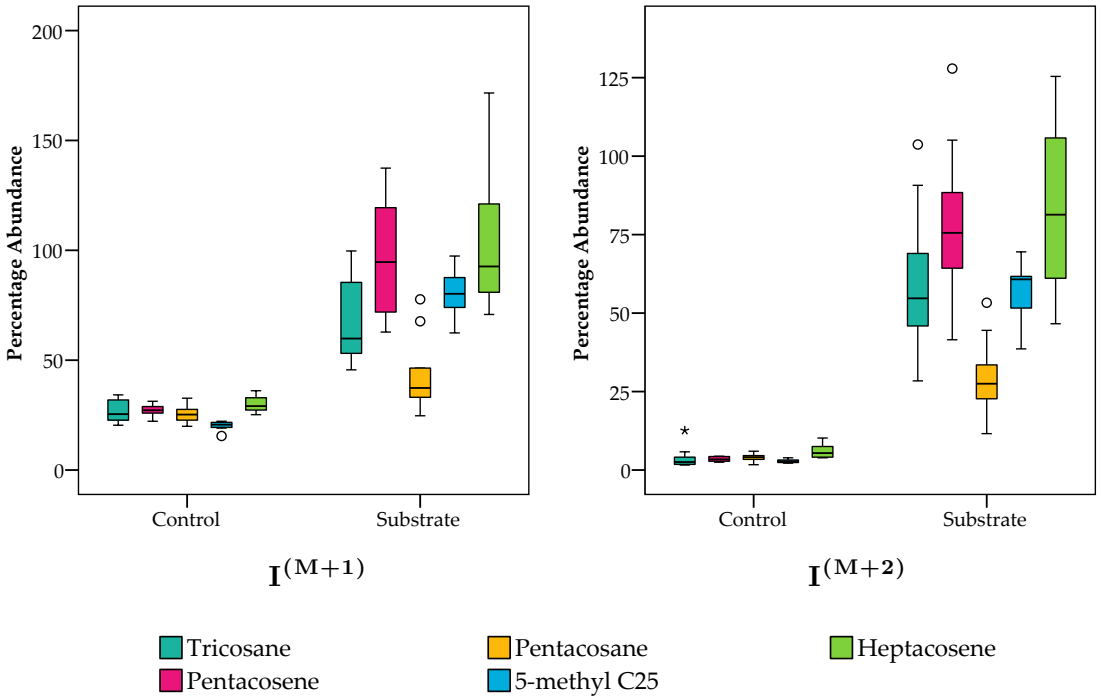


Figure 4.12: Boxplots showing the data for *Myrmica sabuleti* and the substrate sodium [2-^{13}C]acetate; $I^{(M+1)}$ on the left, and $I^{(M+2)}$ on the right.

Again the boxplots show very clear separation between percentage abundances for both $I^{(M+1)}$ and $I^{(M+2)}$. In addition to this, as Figure 4.13 shows there was a very clear difference between the general mass spectra of the two groups. Whilst the appearance of the control spectra was considered 'normal' with a gradual decrease in the ion counts to almost 0 for M+2, for the substrate group no real decrease between isotope levels was observed, with the ion count for M+2 almost equal to that of the molecular ion (shown as M in this Figure). See Figure 4.13.

Figure 4.13: Extracted mass spectrum for *Myrmica sabuleti* and the sodium [2-^{13}C]acetate experiment showing the average comparative ion counts for control and substrate data sets for the compound pentacosene.

The clear difference in experimental groups was confirmed using Mann-Whitney U-tests, which revealed that in all cases p=0.000. See Table 4.3 for a summary of the Mann-Whitney U-test data.

Table 4.3: The probability values from the calculated Mann-Whitney U-tests for *Myrmica sabuleti*, for both substrates, and both isotope peaks.

Compound	p value			
	Sodium [1-^{13}C]acetate		Sodium [2-^{13}C]acetate	
	$I^{(M+1)}$	$I^{(M+2)}$	$I^{(M+1)}$	$I^{(M+2)}$
Tricosane	0.000	0.000	0.000	0.000
Pentacosene	0.000	0.000	0.000	0.000
Pentacosane	0.000	0.000	0.000	0.000
5-methyl C25	0.000	0.000	0.000	0.000
Heptacosene	0.000	0.000	0.000	0.000

4.3.1.4 Discussion

The results from the first of the substrate experiments indicate that *F. lugubris*, *F. lemani* and *M. sabuleti* use sodium acetate during the biosynthesis of alkene, alkane, and methyl-branched hydrocarbons. When the labelled sodium acetate is used during the biosynthesis of these compounds the carbon-13 atoms from the label are incorporated into the carbon chain, and therefore the amount of isotope detected in the resulting mass spectrum is much greater. By measuring the height of the M peak and the height of the M+1 peak, the relative height of the M+1 peak compared to the height of the M peak can be determined and represented as a percentage abundance. The greater percentage abundance values for the substrate fed ants compared to those fed a control diet indicate that the sodium acetate is incorporated into the biosynthesised hydrocarbons.

However although each species was treated in exactly the same way there are clear differences in the levels of incorporation. For the substrate sodium [1-^{13}C]acetate the highest levels of incorporation were seen for *F. lugubris*; this can be seen by comparing the intensities of the isotope peaks at both levels across the species. The observed levels of incorporation are much greater than that observed for *F. lemani* and *M. sabuleti*, but only for the substrate sodium [1-^{13}C]acetate. For the substrate sodium [2-^{13}C]acetate the greatest level of incorporation is seen for *M. sabuleti*. The reasons for this are unknown; however there are several possible explanations. The first is that the differences are not related to the biosynthetic route; i.e. it could be down to food preference, or rate of turnover of the hydrocarbons. If more food was eaten by the *F. lugubris* ants then they would have more labelled substrate to utilise. This could also be related to the physical size of these ants; *F. lugubris* are easily the biggest of these three species and therefore they may require more food to sustain themselves. If they consume more food then this may cause the greater levels of incorporation observed for this species. However based on this argument it might therefore be expected that the next biggest ant species, *F. lemani*, would show more incorporation than *M. sabuleti*, however this

trend is not observed. It would also not be the case that for sodium [2-^{13}C]acetate the greatest incorporation is shown by the smallest ant; *M. sabuleti*. Therefore it is likely that there is a different driving force. It is known that as the size of the ant decreases, the surface area to volume ratio increases, and thus the small size of *M. sabuleti* makes it more prone to desiccation [8]. One of the main purposes of the cuticular hydrocarbons in insects is to prevent dessication via the evaporation of water [9–11], therefore because a smaller insect is more likely to lose water the production and rate of turnover of the cuticular hydrocarbons may be greater in smaller insects. This may explain the elevated incorporation seen for the smaller *Myrmica sabuleti* compared to *F. lemani*. In this case the smaller ant would therefore have a faster rate of hydrocarbon production and therefore show more elevated levels of incorporation as more of the labelled substrate would be required. It may therefore be that the levels of incorporation seen for these species is related both to the amount of food consumed and the rate of cuticular hydrocarbon production.

Although size and hydrocarbon production may be key factors in the levels of incorporation biosynthetic factors should also be considered. It has been established that sodium acetate is used to lengthen the fatty acid precursor [6] and thus is incorporated into the final hydrocarbon, however the variation may be down to subtle differences in the precursors that can be used. Within nature many fatty acids exist with differing numbers of carbons in their chain length. The length of the initial fatty acid used as a precursor will dictate the number of acetate units that are added in order to reach the desired chain length and hence the level of incorporation. For example in order to make the hydrocarbon pentacosane, five acetate units are required if the starting material is hexadecanoic acid, whereas four are required if the starting material is octadecanoic acid. If a greater number of acetates are used then the chances of incorporating a labelled one are greatly increased. However in order to determine whether this is the case more experimentation is needed with additional labelled substrates.

As previously mentioned these substrates differed only in the position of the label. One substrate had the label on the first carbon in the chain whilst the other had the label on the second carbon in the chain. The data shows that both of these substrates were incorporated regardless of the position of the label. This suggests that the acetate molecule is not modified or broken apart as both carbons were included into the final biosynthesised hydrocarbon. According to the postulated biosynthetic routes shown in Chapter 1, the acetate units can be used in a variety of different ways, however these results suggest that they are likely used in the final hydrocarbon with the two carbon chain length intact. The fact that both substrates were found to be incorporated strengthens the assumption that this biosynthetic route is correct.

Analysis of the literature prior to this experiment revealed that the use of labelled acetate compounds is fairly widespread [4, 7, 12]. In these studies the researchers used sodium [1-^{14}C]acetate and injected small volumes directly into the haemolymph of a variety of ant species (*Cataglyphis niger* and *Pachycondyla apicalis* [4, 7, 12]). However in these studies the use of labelled hydrocarbons was a means to track the distribution and transportation of hydrocarbons through the system from the site of synthesis, rather than as a means of elucidating biosynthetic routes.

Current theory on the biosynthesis of hydrocarbons suggests that the incorporation of labelled acetate units could occur at two different points during the biosynthesis of long chain hydrocarbons. Firstly these acetate units can be used in the *de novo* synthesis of fatty acids, whereby acetate is first converted to malonate [6], see Section 1.2.2.2 for further information. Long chain fatty acids such as stearic acid can also be obtained through diet. If this is the case the length of the acid must be extended in order to reach a required length, which is one carbon longer than the desired long chain alkene or alkane. Again in this case acetates which are first converted to malonate are used; the addition of each acetate increases the chain length by two carbons [6]. See Section 1.2.2.3 for further details on this process.

The use of labelled substrates as indicators for possible biosynthetic routes is a technique that has been used with varying success for other species. A study in 1989 published by Chase et al. [2] involved injecting cockroaches with a variety of labelled substrates including potassium [1-^{13}C]acetate and sodium [2-^{13}C]acetate. The insects were injected daily and extracted after 18 days; these extracts were pooled and ^{13}C NMR was performed. The results of this study showed that both compounds were incorporated, however the position of the enriched carbons varied depending on the substrate that was used. The authors state that the first carbon in the resulting hydrocarbon chain was enriched by the [2-^{13}C]acetate and the second carbon by the [1-^{13}C]acetate; from this they suggested that the acetate group is used during the initial step of the synthesis of cuticular hydrocarbons.

In a similar study by Dillwith [3] the authors studied the biosynthesis of methyl-branched hydrocarbons in the house fly. Again the authors of this study used sodium [1-^{13}C] and [2-^{13}C]acetate, however they fed these substrates as a mixture with sugar solution and milk powder. As with the aforementioned study by Chase et al. [2], the [1-^{13}C]acetate labelled the even carbons within the chain, and the [2-^{13}C]acetate the odd. Based on the results of these studies and the results shown above it can therefore be assumed likely that the [1-^{13}C]acetate used in this study is labelling the even carbons. Therefore it would be expected that the same work with sodium [2-^{13}C]acetate would show greater incorporation as there are more odd numbered carbons within our species. In terms of the results seen above this theory supports the data. For all species there appears to be more incorporation for the sodium [2-^{13}C]acetate, this can be seen by comparing the raw percentage substrate values against the controls, see Figures B.1 to B.11, Appendix B. The reason for this could be that suggested above. However without accurate quantification work it is not possible to establish this conclusively. It is worth considering whether these two studies are comparable; they were performed at different times with different groups of ants. Therefore varying temperatures or conditions, as well as differing ant populations between the experiments may affect the

rates of feeding and thus the amount of incorporation seen.

It may be assumed that the work done on the above mentioned species was unnecessary given that it is already known that acetates are used during the biosynthesis of long chain hydrocarbons. However the results shown by these experiments confirmed that this theory and basis holds true for our specific species. It also confirmed that these species are suitable for this kind of work and allowed the experimental and analytical procedure to be rigorously tested. Based on these results, and observations made throughout, changes and refinements were made for the next round of experimentation.

4.3.2 Preliminary propionate experiment

As well as acetate compounds propionates are also widely thought to be used during the biosynthesis of hydrocarbons. This preliminary experiment repeated the same procedure with the propionate substrates sodium [1-^{13}C]propionate and propionic-2,2-d_2 acid-d. The same three ant species of *Formica lugubris*, *Formica lemani* and *Myrmica sabuleti* were used, however the number of samples and studied compounds was modified. For full details of the procedure see Section 4.2.2.

4.3.2.1 *Formica lugubris*

Despite the poor results that were obtained for this species with the acetate experiment it was decided to persevere with this species. However at the end of the 30 day period there were very few samples for the sodium [1-^{13}C]propionate. Therefore only the results for propionic-2,2-d_2 acid-d are presented. For this set of data n=14 for the control group and n=18 for the substrate group.

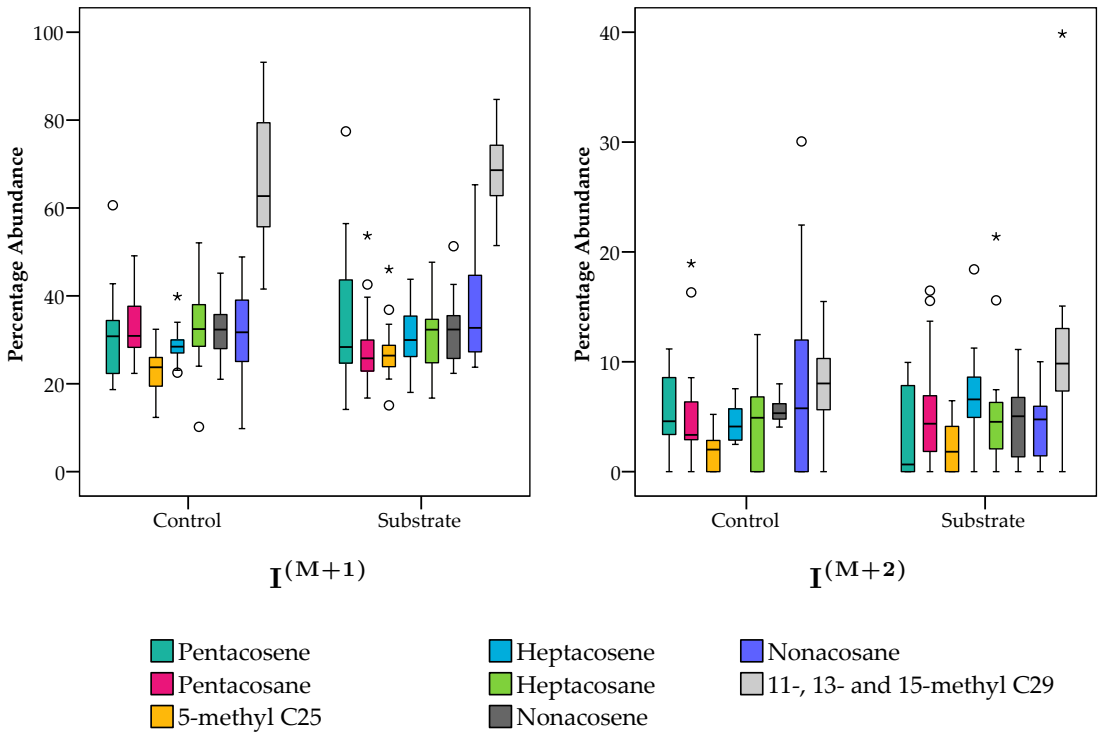


Figure 4.14: Boxplots showing the data for *Formica lugubris* and the substrate propionic-2,2-d_2 acid-d; $I^{(M+1)}$ on the left, and $I^{(M+2)}$ on the right.

As Figure 4.14 shows there is very little observable difference in the median ranks for each group. Generally there is a similar range for the data, indicating a consistency across the experiment. However as the asterisks within the plots indicate there are several extreme outliers within the data sets. The associated p values are given in Table 4.4.

Table 4.4: The probability values from the Mann-Whitney U-tests for *Formica lugubris*, for propionic-2,2-d_2 acid-d, and both isotope peaks. Note that the p value for pentacosane for $I^{(M+1)}$ appears to be significant, however this is based on the median rank of the control group being higher than the substrate group, and is therefore disregarded, all future values of this type will be represented in italics.

Compound	p value	
	Propionic-2,2-d_2 acid-d	
	$I^{(M+1)}$	$I^{(M+2)}$
Pentacosene	0.492	0.189
Pentacosane	*0.023*	0.262
3-methyl C25	0.120	0.484
Heptacosene	0.389	0.059
Heptacosane	0.253	0.390
Nonacosene	0.418	0.292
Nonacosane	0.256	0.371
11-, 13-, and 15-methyl C29	0.293	0.960

Figure 4.14 also shows an interesting feature that was seen across several data sets. The boxplot for propionic-2,2-d_2 acid-d $I^{(M+1)}$ (on the left) shows that the range of data and the median value is much greater for the co-eluting 11-, 13- and 15-methylnonacosane plot, shown in pale grey, compared to the plots for other compounds. This is a trend that was seen across several sets of data but only for these co-eluting, medially positioned, methyl-branched compounds. It can also be seen that this trend is repeated in the substrate group but again only for these compounds, and also only for the $I^{(M+1)}$ data. The reason for this considerable increase is due to the fragment masses that are measured. Within the mass spectrometer the carbon-carbon bond of the methyl-

branch of these compounds is easily cleaved, which means that rarely is the whole ionised compound detectable. This means that measuring the isotopic incorporation using the molecular ion of methyl-branched compounds is inaccurate due to the associated low abundance. Instead, for methyl-branched compounds, key fragment masses were selected, which were then used for the incorporation measurements, see Figure 3.9 for a schematic showing the key ions for the various measured compounds and species. For 11-, 13- and 15-methylnonacosane, the fragment 196 *m/z* was selected as it was generally present in high abundance. However unlike the fragments selected for other compounds, 196 is an even number. Generally the masses of these fragments should be odd numbers; however hydrogens are easily lost from these fragments and a even mass number fragment, such as 196, is the result. This essentially means that, in this case, M+1 of the 196 *m/z* fragment can be caused by isotopic incorporation, or the entire fragment, without the loss of a hydrogen, reaching the mass detector. Therefore because there are two routes resulting in a fragment of 197 i.e. M+1 of 196 *m/z*, the percentage values are much higher for this fragment. However it should be noted that all analysis compared like for like, control to substrate values, so even though this value is higher compared to other compounds, this has no effect on the analysis.

As can be seen from this, at the conclusion of this experiment there was no difference in the percentage abundances between the control and the substrate groups. Note that for the statistical analysis of this data a one-tailed value of Z was used as the power of the analysis was directed only towards identifying whether isotope levels are greater for the substrate compared to the control. However, on occasion, statistical analysis reveals the control values to be significantly greater than the substrate values. Therefore any p values which indicate significance due to the control being greater than the substrate will be shown in italics within the probability value tables.

4.3.2.2 *Formica lemani*

For the results of this experiment please see Figures 4.15 and 4.16. Note that for this experiment and for the subsequent experiments all ants remaining at the end of the experiment were extracted therefore for this data set n=20 for the control, n=19 for sodium [1-^{13}C]propionate and n=18 for propionic-2,2-d_2 acid-d.

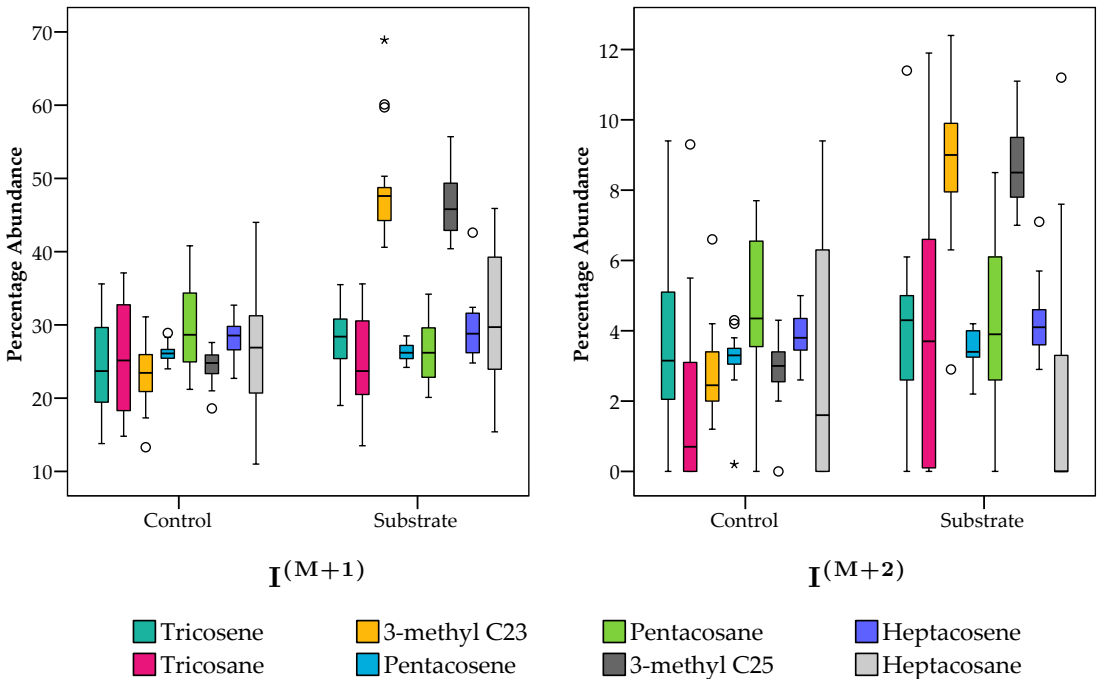


Figure 4.15: Boxplots showing the data for *Formica lemani* and the substrate sodium [1-^{13}C]propionate; $I^{(M+1)}$ on the left, and $I^{(M+2)}$ on the right.

As Figure 4.15 shows, for the methyl-branched compounds, 3-methyltricosane and 3-methylpentacosane, shown in yellow and dark grey respectively, there is a clear difference in the median ranks between the substrate and the control, which is consistent across the different isotope peaks M+1 and M+2. For the alkene and alkane compounds there appears to be no difference in the percentage abundances.

Figure 4.16 shows the data for propionic-2,2-d_2 acid-d. As can be seen there is far less variation in the median ranks between the two groups. The range of the data is also much smaller. However the main difference between Figures 4.15 and 4.16 can be seen

when looking at the percentage abundance for the methyl-branched compounds. The results for sodium [1-^{13}C]propionate showed clear incorporation, with a large difference in percentage abundance for the methyl-branched compounds. However for propionic-2,2-d_2 acid-d there is no clear difference observable between the samples.

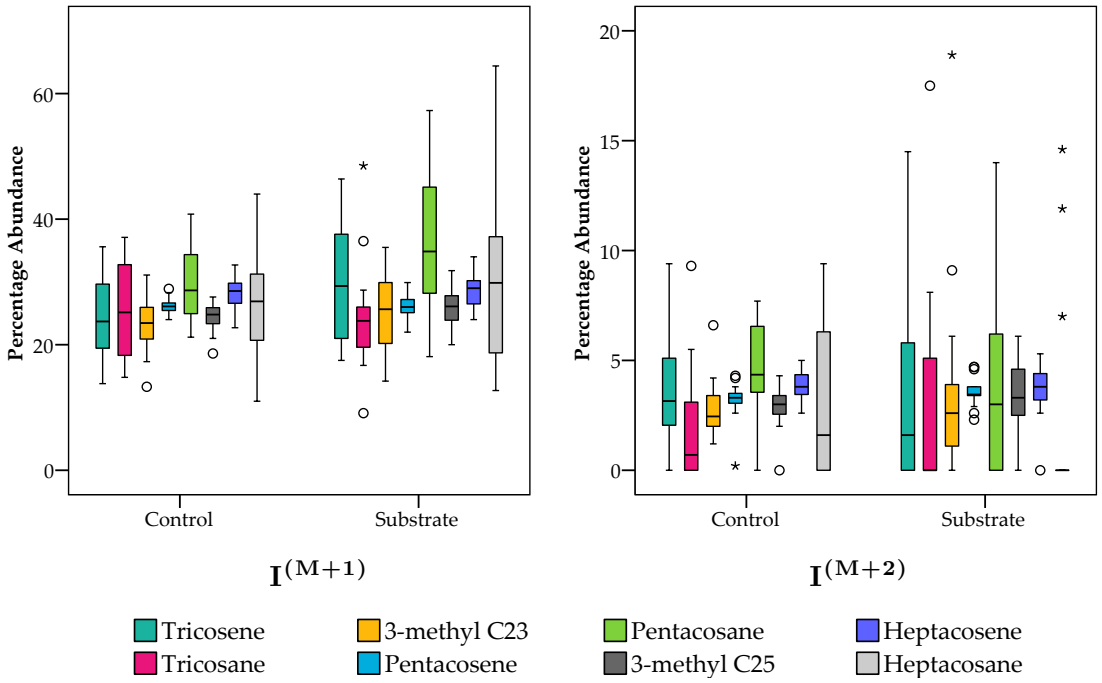


Figure 4.16: Boxplots showing the data for *Formica lemani* and the substrate propionic-2,2-d_2 acid-d; $I^{(M+1)}$ on the left, and $I^{(M+2)}$ on the right.

To confirm any statistical differences Mann-Whitney U-tests were performed and Table 4.5 shows the combined results of these calculations.

Table 4.5: The probability values from the calculated Mann-Whitney U-tests for *Formica lemani*, for both substrates, and both isotope peaks. Any p values less than 0.050 which are italicised may be disregarded and are due to the median rank of the control being greater than that of the substrate group.

Compound	p value			
	Sodium [1-^{13}C]propionate		Propionic-2,2-d$_2$ acid-d	
	$I^{(M+1)}$	$I^{(M+2)}$	$I^{(M+1)}$	$I^{(M+2)}$
Tricosene	*0.047*	0.287	0.028	0.055
Tricosane	0.481	0.021	0.158	0.315
3-methyl C23	0.000	0.000	0.219	0.307
Pentacosene	0.470	0.073	0.316	0.320
Pentacosane	0.360	0.306	0.044	*0.004*
3-methyl C25	0.000	0.000	0.057	0.156
Nonacosene	0.372	0.199	0.111	0.093
Nonacosane	0.039	0.112	0.106	0.096

When looking at the boxplots for sodium [1-^{13}C]propionate it can be seen that there is a clear difference in the median ranks for the two studied methyl-branched compounds. This is supported by the p values which for these compounds are 0.000. However the Mann-Whitney calculations also indicate significance for nonacosane, although this value is greater at 0.039. It should also be noted that the Mann-Whitney results show significance for tricosene, however this is due to the percentage abundances being greater for the control group compared to the substrate group, an anomaly sometimes encountered when the two groups are very similar. These calculations also indicate that there is statistical difference within the propionic-2,2-d$_2$ acid-d data for tricosene and pentacosane (p=0.028 and 0.044 respectively).

This data clearly shows that *Formica lemani* are able to use sodium [1-^{13}C]propionate to biosynthesise methyl-branched hydrocarbons. The p values of 0.000 for the two methyl-branched hydrocarbons indicate that there is a clear difference in the percentage abundances of the isotope peaks for these compounds, between the group fed on the labelled substrate, and the control group. However as Table 4.5 shows there are

other compounds which also show statistical significance. The p values in these cases are much greater, therefore any difference in the groups is much more subtle. Based on this data it is not possible to determine whether there is clear incorporation of the labelled substrate into straight chained hydrocarbons as well as methyl-branched ones. The same can also be said for the propionic-2,2-d_2 acid-d results. There are p values which are ≤ 0.050, however again it cannot be said with certainty that there is clear incorporation, this is mainly because clear incorporation should result in statistical significance at both levels, i.e.similar to that seen for the methyl-branched compounds and sodium [1-^{13}C]propionate. Without incorporation at both measured levels it is impossible to say that this is clear incorporation, however there is no doubt that the statistical analysis reveals that there may be some incorporation of the labelled substrates into straight chain compounds.

4.3.2.3 *Myrmica sabuleti*

For the results of this experiment please see Figures 4.17 and 4.19. Note that for this data set n=20 for the control, n=24 for sodium [1-^{13}C]propionate and n=21 for propionic-2,2-d_2 acid-d.

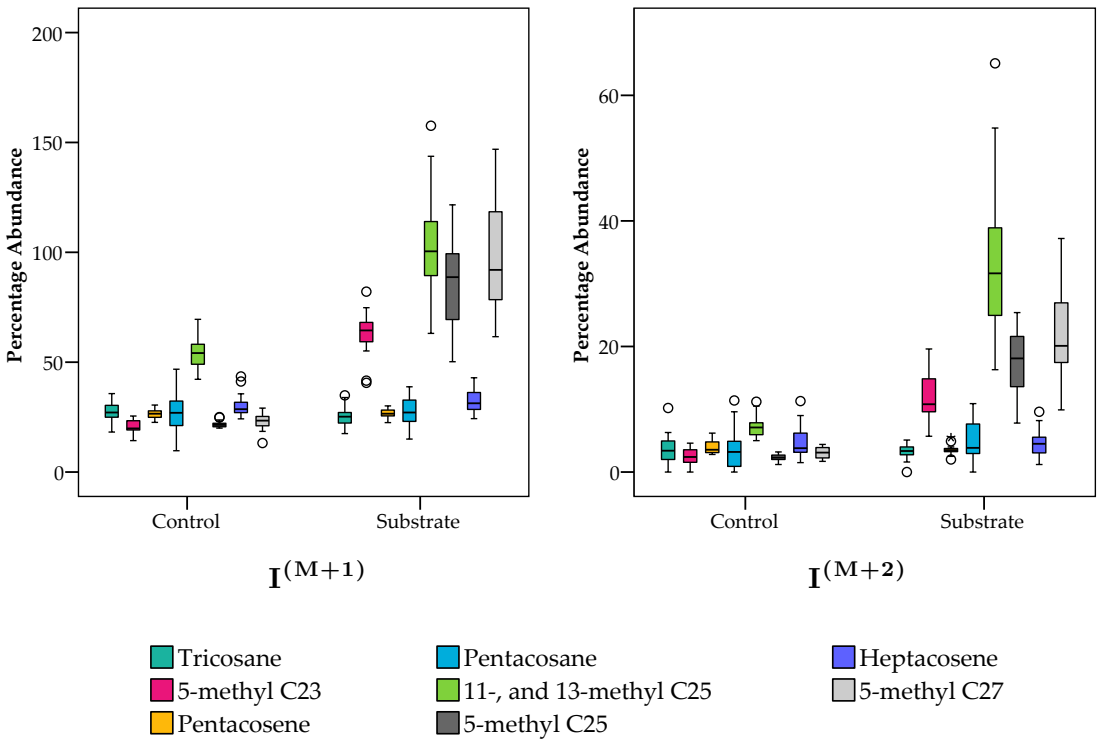


Figure 4.17: Boxplots showing the data for *Myrmica sabuleti* and the substrate sodium [1-^{13}C]propionate; $I^{(M+1)}$ on the left, and $I^{(M+2)}$ on the right.

The boxplots for sodium [1-^{13}C]propionate $I^{(M+1)}$ and $I^{(M+2)}$ show that again there is a clear difference in the median ranks for the four methyl-branched compounds, these are shown by the pink, green, and grey (light and dark) boxes. The data as a whole has a fairly small range and is consistent between $I^{(M+1)}$ and $I^{(M+2)}$ measurements, with the same trends observed in each.

Figure 4.18 shows the comparative mass spectra for the control and sodium [1-^{13}C]propionate group for the methyl branched compound 5-methyl pentacosane. As can be seen from this Figure the ion count for M+2 is elevated compared to the control equivalent. The mass spectra for the other methyl compounds showed a similar appearance to this extract, whilst the straight chain compounds were conidered similar in appearance to the control.

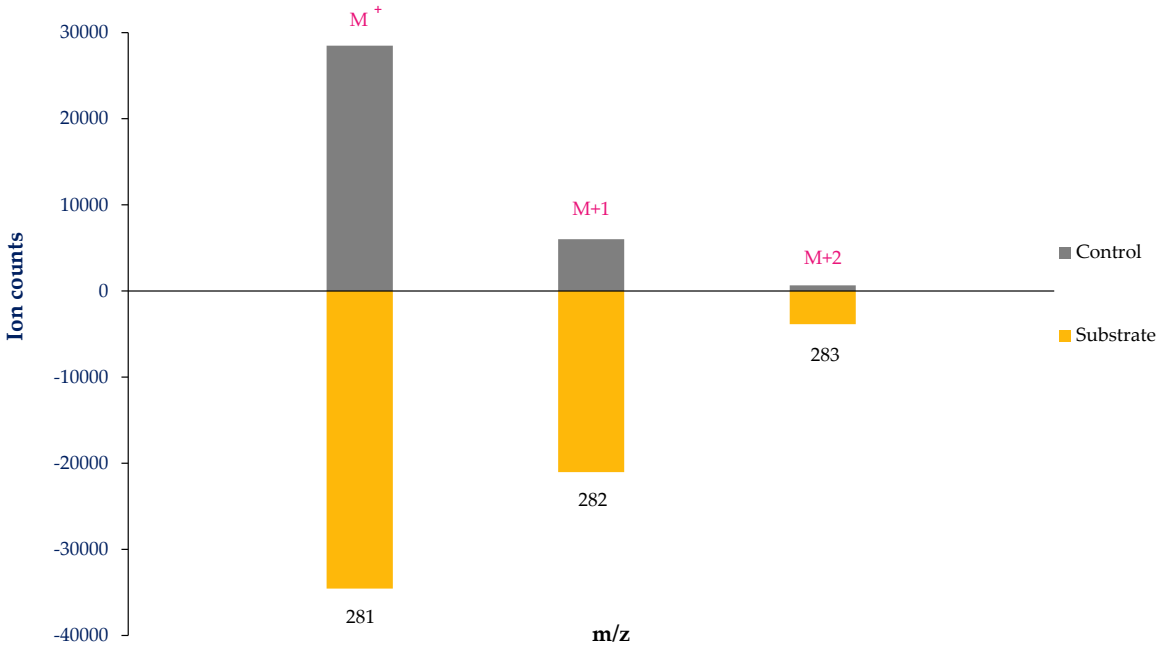


Figure 4.18: Extracted mass spectrum for *Myrmica sabuleti* and the sodium [1-^{13}C]propionate experiment showing the average comparative ion counts for control and substrate data sets for the compound 5-methyl pentacosane.

The boxplots for propionic-2,2-d$_2$ acid-d $I^{(M+1)}$ and $I^{(M+2)}$, shown in Figure 4.19, indicate that there does not appear to be the same clear difference in the median ranks between the substrate and control groups for the same methyl-branched compounds; possible reasons for this will be explored in the discussion. In addition, the data for $I^{(M+2)}$ has a much greater spread of values compared to that of $I^{(M+1)}$.

Looking at Table 4.6, the p values for sodium [1-^{13}C]propionate indicate, as expected, that there is clear statistical difference for the four methyl-branched compounds for both measured isotope peaks, with these compounds having p values of 0.000. There is also a statistically significant result for heptacosene (p=0.048), however less confidence can be gained from this as it is only observed for $I^{(M+1)}$, and it is only just below the critical p value of 0.050. The Mann-Whitney propionic-2,2-d$_2$ acid-d results show the same pattern in that the same methyl-branched hydrocarbons show a clear statistical difference for both measured isotope peaks. There is also the same statistical difference for heptacosene (p=0.007), but again only for $I^{(M+1)}$. Overall the results of this

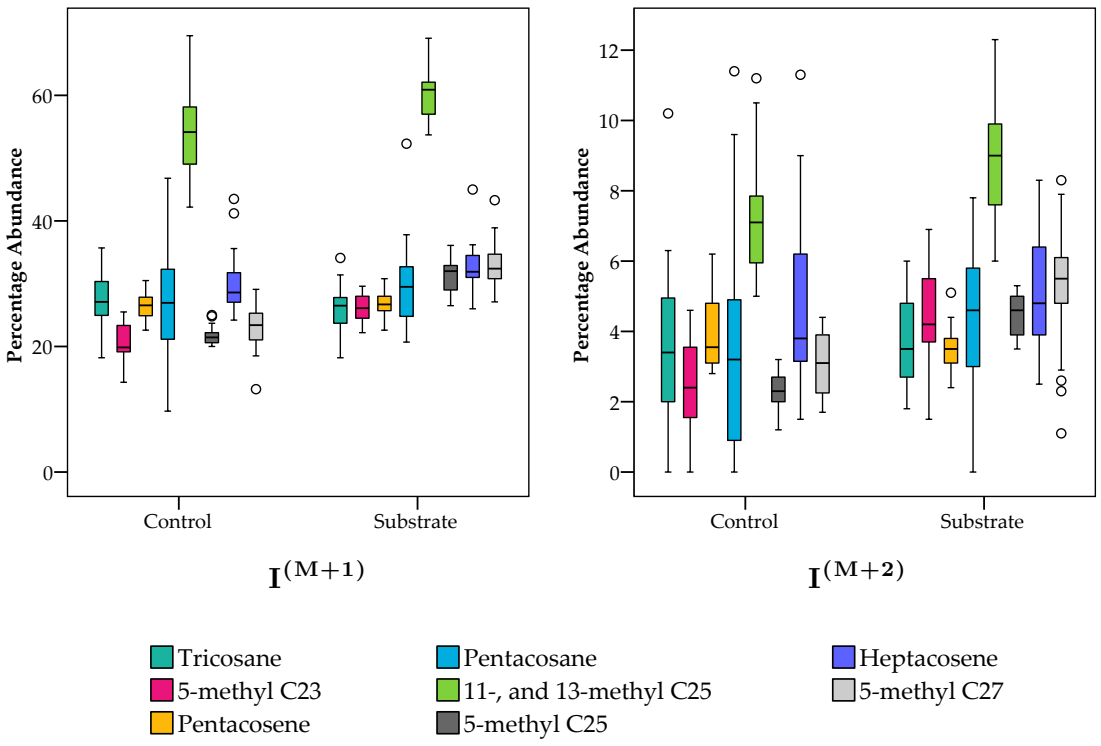


Figure 4.19: Boxplots showing the data for *Myrmica sabuleti* and the substrate propionic-2,2-d_2 acid-d; $I^{(M+1)}$ on the left, and $I^{(M+2)}$ on the right.

Table 4.6: The probability values from the calculated Mann-Whitney U-tests for *Myrmica sabuleti*, for both substrates, and both isotope peaks.

Compound	p value			
	Sodium [1-^{13}C]propionate		Propionic-2,2-d_2 acid-d	
	$I^{(M+1)}$	$I^{(M+2)}$	$I^{(M+1)}$	$I^{(M+2)}$
Tricosane	0.072	0.352	0.171	0.361
5-methyl C23	0.000	0.000	0.000	0.000
Pentacosene	0.465	0.236	0.406	0.228
Pentacosane	0.392	0.062	0.134	0.081
11-, and 13-methyl C25	0.000	0.000	0.002	0.001
5-methyl C25	0.000	0.000	0.000	0.000
Heptacosene	0.048	0.396	0.007	0.188
5-methyl C27	0.000	0.000	0.000	0.000

experiment indicate that when *Myrmica sabuleti* are fed sodium [1-^{13}C]propionate and propionic-2,2-d_2 acid-d they are able to use both propionate compounds during the biosynthesis of their methyl-branched hydrocarbons.

4.3.2.4 Discussion

The results obtained from *Formica lugubris* were disappointing with only partial results obtained and no statistical significance observed. However the results for *Formica lemani* and *Myrmica sabuleti*, the other two species, were much more interesting. Both species indicated clear incorporation of sodium [1-^{13}C]propionate into their methyl-branched hydrocarbons. This suggests that the groups of ants of these species fed sodium [1-^{13}C]propionate used this labelled propionate compound during the biosynthesis of their methyl-branched hydrocarbons. Again this was a largely expected result as the literature describes how previous studies have shown that propionates are used in the biosynthesis of methyl-branched hydrocarbons. As described in Chapter 1, methyl-branches are introduced via the use of propionates (as methylmalonate), instead of acetates at the relevant point of the chain extension, which is dependant on the position of the methyl-branch to be inserted [6]. See Section 1.2.2.4 and Figure 1.15 for a more thorough explanation of this process. This explains why high levels of incorporation are seen only for methyl-branched hydrocarbons.

However the data also shows that labelled atoms are being incorporated into some of the straight chain hydrocarbons. In 1982, a study by Dillwith et al. showed the labelled ^{13}C of [2-^{13}C] and [3-^{13}C]propionate in the ^{13}C NMR spectra of straight chain alkenes from the CHCs of housefly *Musca domestica*. On this basis they concluded that within this species propionates can also be metabolised to form acetates, and therefore used in the biosynthesis of straight chain alkenes and alkanes. Based on the data shown above it appears that these species of ants are also capable of metabolising propionates to form acetates. Further experimentation was performed by researchers in this area and it was hypothesised that propionates are metabolised to acetates via the route shown in Figure 4.20 [6, 8, 13]. The results shown for this experiment support this finding as the lower percentage abundance values observed for the straight chain compounds compared to methyl-branched compounds, suggest this to be a minor biosynthetic pathway.

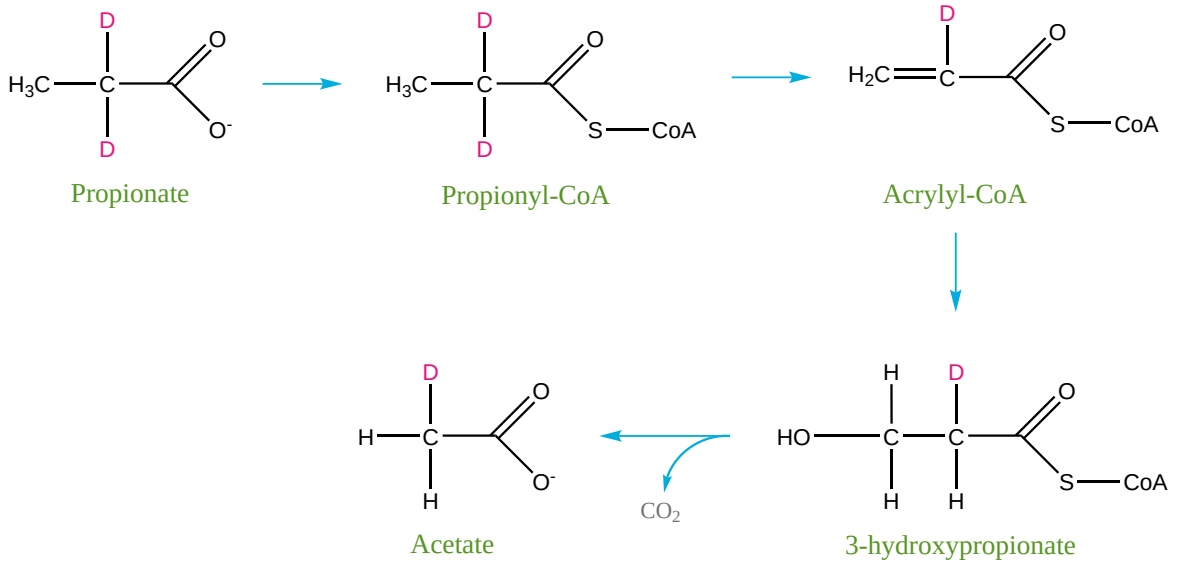


Figure 4.20: Schematic showing the hypothesised metabolism of the labelled propionate into the resulting labelled acetate. Adapted from [13].

When comparing the results for the propionic-2,2-d_2 acid-d substrate for *Formica lemani* and *Myrmica sabuleti* it can be seen that there is a difference in the result observed for each of the two species. For *F. lemani* there is no clear incorporation of this substrate, however for *M. sabuleti* clear incorporation is observed. Again the incorporation is only seen for the methyl-branched hydrocarbons for both M+1 and M+2 and again it is very clear, in all cases $p \leq 0.020$. Whilst it was no surprise that the sodium [1-^{13}C]propionate was incorporated, the incorporation of the propionic-2,2-d_2 acid-d substrate was more unexpected. The two tested substrates differed in the position of the label, in that for sodium [1-^{13}C]propionate the label is on the first carbon, and for propionic-2,2-d_2 acid-d there are two deuterium labels attached to the second carbon. In the hypothesised mechanism of the incorporation of [1-^{13}C]propionate by Blomquist [8] the carbon-13 label in the one position becomes the carbon adjacent to that which the branch originates from, i.e. carbon four for a 3-methyl-branched compound, see Figure 4.21. As this figure shows, a labelled carbon in the one position is incorporated into a methyl-branched hydrocarbon at the adjacent carbon site.

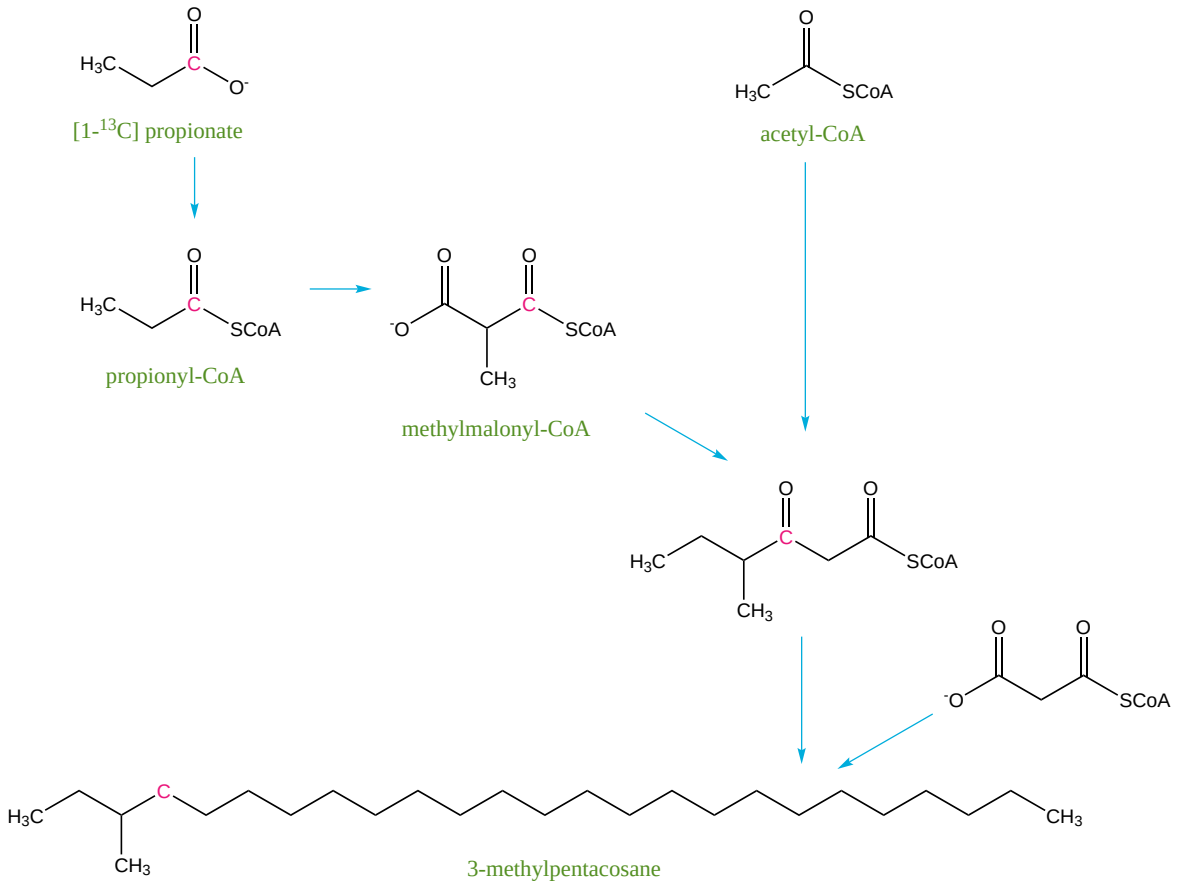


Figure 4.21: Diagram showing the process of incorporation of [1-^{13}C]propionate into 3-methylpentacosane. Note the position of the ^{13}C label is shown in pink. Adapted from [8].

Propionic-2,2-d_2 acid-d differed slightly in that, due to cost, deuterium labels were used instead of labelled carbons. However the principle was the same in that the label was centred on the second carbon in the chain. According to the same proposed mechanism by Blomquist [8] the initial step in the proposed biosynthesis of methyl-branched hydrocarbons is the formation of methylmalonate. During this initial step one of the deuterium atoms is lost from the second carbon. During this step the deuterium on the acid group is also lost. See Figure 4.22 for reference.

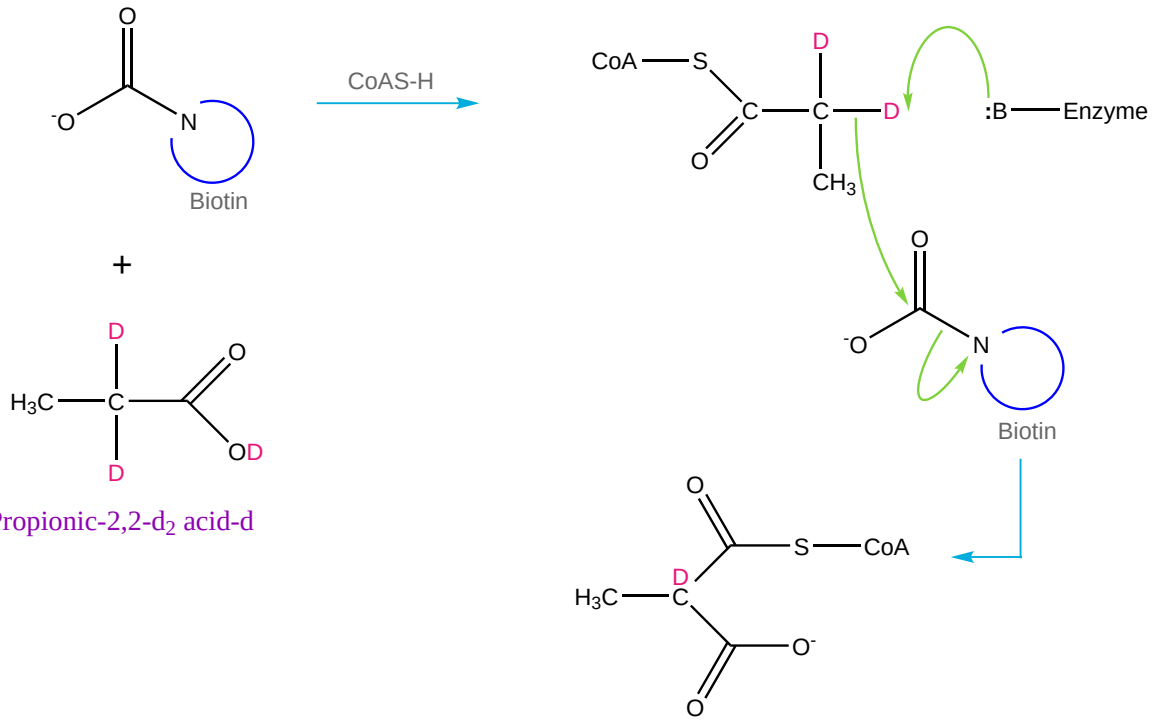


Figure 4.22: Proposed mechanism for the formation of methylmalonate from propionic-2,2-d_2 acid-d, adapted from [8]. As indicated from this mechanism, only one deuterium label is incorporated into the methylmalonate molecule.

When this proposed mechanism is followed through however, it is seen that the final deuterium label is removed when the chain is extended [6]. See Figure 4.23.

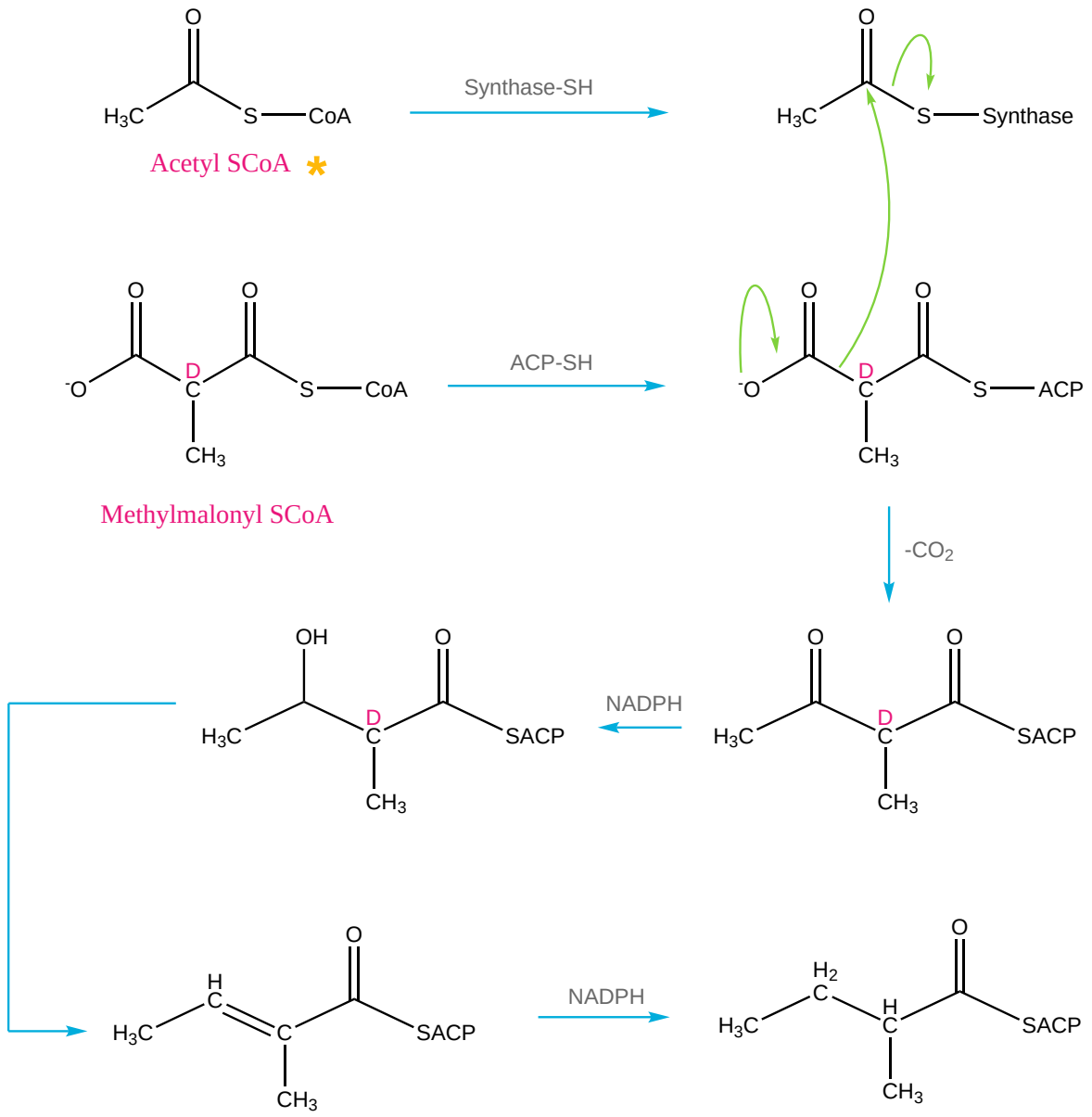


Figure 4.23: Proposed mechanism for the formation of a fatty acid using methylmalonate via propionic-2,2-d_2, adapted from [8]. As can be seen, according to this mechanism, the final deuterium label is lost towards the end of the biosynthesis during the chain lengthening steps.

If this mechanism is indeed used during the synthesis of methyl-branched compounds then it explains why the extra mass of the deuterium is not detected within the hydrocarbons of *Formica lemani* and *Formica lugubris*. However in the same fashion it does not explain why the extra mass is detected for *M. sabuleti*. The extra mass is only one unit, which suggests that it is only one deuterium atom reaching the final hydrocarbon, however its origin is uncertain.

The main issue with this substrate is that deuterium is not detectable via standard NMR studies, and therefore it is much harder to determine the location of the deuterium atom. Because there is a statistical difference between the control and propionic-2,2-d_2 acid-d for *M. sabuleti*, it can only be assumed that there is a different, unknown mechanism to that previously suggested. This mechanism applies not only to 3-methyl-branched hydrocarbons but also to internally branched hydrocarbons (11-, and 13-methyl). This result is based on a single experiment, however there was a high number of repeats; 21 individual ants were sampled to ensure a thorough analysis. Although the results are less statistically different than those seen for sodium [1-^{13}C]propionate, the results of the Mann-Whitney calculations still show them to be significantly different from the control, in all cases $p \leq 0.020$. It could be that this unknown mechanism is a minor route, and hence the reason why the results are less clear. It could be suggested that in time *Formica lemani* may also have shown this same result, but an experimental duration of 30 days was not long enough. As this is an unexpected result further research was performed in this area.

4.3.3 Propionate experiment

The results from the preliminary propionate experiment indicated that clear incorporation was seen for the methyl-branched hydrocarbons of *Myrmica sabuleti* for both propionate compounds. Based on this, and the fact that *Myrmica sabuleti* had generally shown the most consistent results with sufficient levels of hydrocarbons for analysis, it was decided to expand the previous experiment whilst focussing on *Myrmica* species. The next experiment was therefore expanded to use two *Myrmica* species; *Myrmica sabuleti* and *Myrmica scabrinodis*. In addition the number of studied compounds was reduced and intra-experiment repeats were introduced. See Section 4.2.3 for further detail.

There were two key differences with this procedure; the first was that a second control was introduced. As well as a propionic acid control, a second control of sodium [1-^{13}C]acetate was used. This was so that the success of the feeding experiment could be judged. By introducing this control, a substrate which is known to yield positive results, the feeding response of the ants could be determined. As such, this control would be used purely to assess whether feeding had taken place within this control population. Therefore whilst measurements were taken from this data to determine the extent of feeding the numbers will not be presented. However all raw data sheets associated with these experiments can be found within Appendix B.

The other key difference with this procedure was that there was a greater number of intra-experiment repeats, i.e. for each of the three groups; substrate and both controls there were five replicate boxes, each containing around 30 ants. In terms of the data handling, the data for the control groups was combined to form one large sample group, whereas the substrate data was firstly processed treating it separately; and comparing each repeat to the large control groups and then combining all the substrate data to form one large group. The extra number of intra-experiment repeats was included to check whether it was possible that some groups would show much higher incorporation

than others. Analysis suggested that this was not the case and therefore the data presented here shows the results when the individual substrate groups were combined to form one large group, as it was found that combining the repeats made no difference to the interpretation of the results. Note that the sodium [1-^{13}C]acetate control data indicated that successful feeding had taken place.

4.3.3.1 *Myrmica sabuleti*

When the data from the individual boxes was combined n=80 for propionic-2,2-d_2 acid-d, n=42 sodium [1-^{13}C]acetate control and n=73 for the propionic acid control (unlabelled).

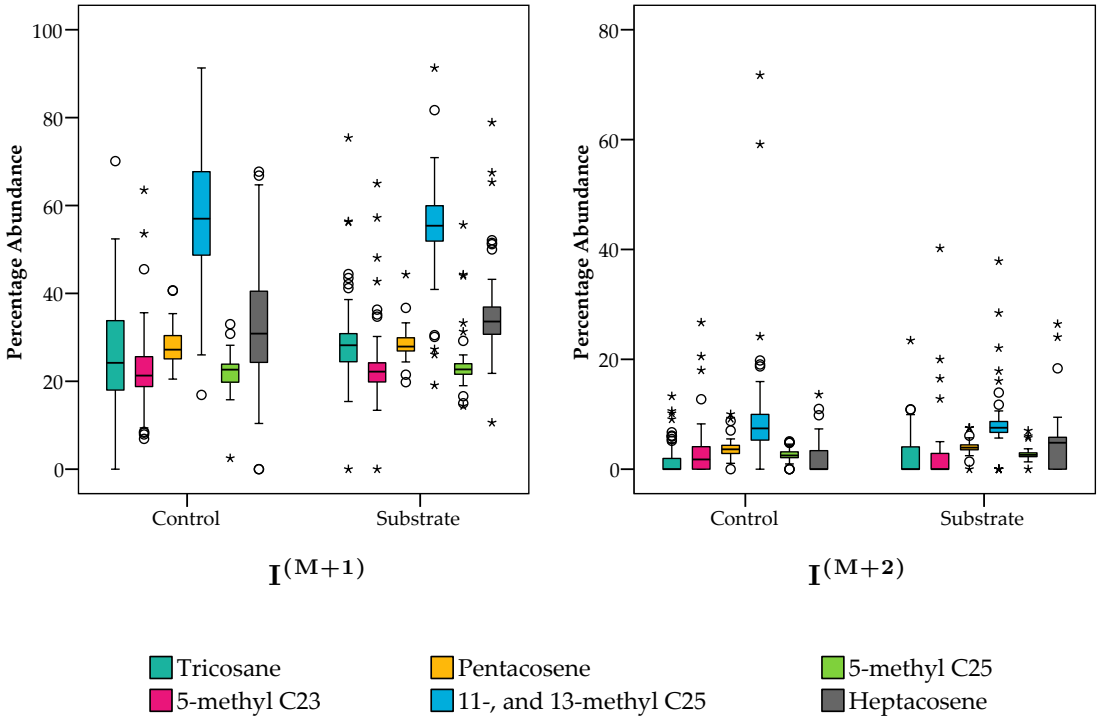


Figure 4.24: Boxplots showing the data for *Myrmica sabuleti* and the substrate propionic-2,2-d_2 acid-d; $I^{(M+1)}$ on the left, and $I^{(M+2)}$ on the right.

Figure 4.24 shows that, because the group sizes are so big, there are many outliers and extreme outliers. Looking at the data presented there does not appear to be any clear

difference between the groups. This is in contrast to the results presented previously and shown in Figure 4.19. In order to further analyse the data Mann-Whitney U-tests were performed.

Table 4.7: The probability values from the Mann-Whitney U-tests for *Myrmica sabuleti*, for propionic-2,2-d_2 acid-d, and both isotope peaks.

Compound	p value	
	Propionic-2,2-d_2 acid-d	
	$I^{(M+1)}$	$I^{(M+2)}$
Tricosane	0.078	0.044
5-methyl C23	0.442	0.217
Pentacosene	0.152	0.047
11-, and 13-methyl C25	0.279	0.205
5-methyl C25	0.072	0.160
Heptacosene	0.104	0.000

For the combined data there is some statistical variation between the labelled propionic acid and the unlabelled propionic acid which serves as a control. The statistical difference between the control and the substrate is seen only for a few compounds and only for the $I^{(M+2)}$ values. This is in contrast to the previous time when this experiment was run when a very clear statistical difference was seen between the control and the substrate for the methyl-branched hydrocarbons. See Table 4.6, which shows the results from the previous experiment.

As these results show, the initial result seen in Section 4.3.2.3 was not observed again. A full analysis of these results can be found in the discussion section.

4.3.3.2 *Myrmica scabrinodis*

Whilst repeating the propionic-2,2-d_2 acid-d substrate with *Myrmica sabuleti* it was decided to try the same experiment with *Myrmica scabrinodis*. For this experiment there were fewer samples; n=50 for propionic-2,2-d_2 acid-d, n=61 sodium [1-^{13}C]acetate

control and n=58 for the propionic acid control (unlabelled).

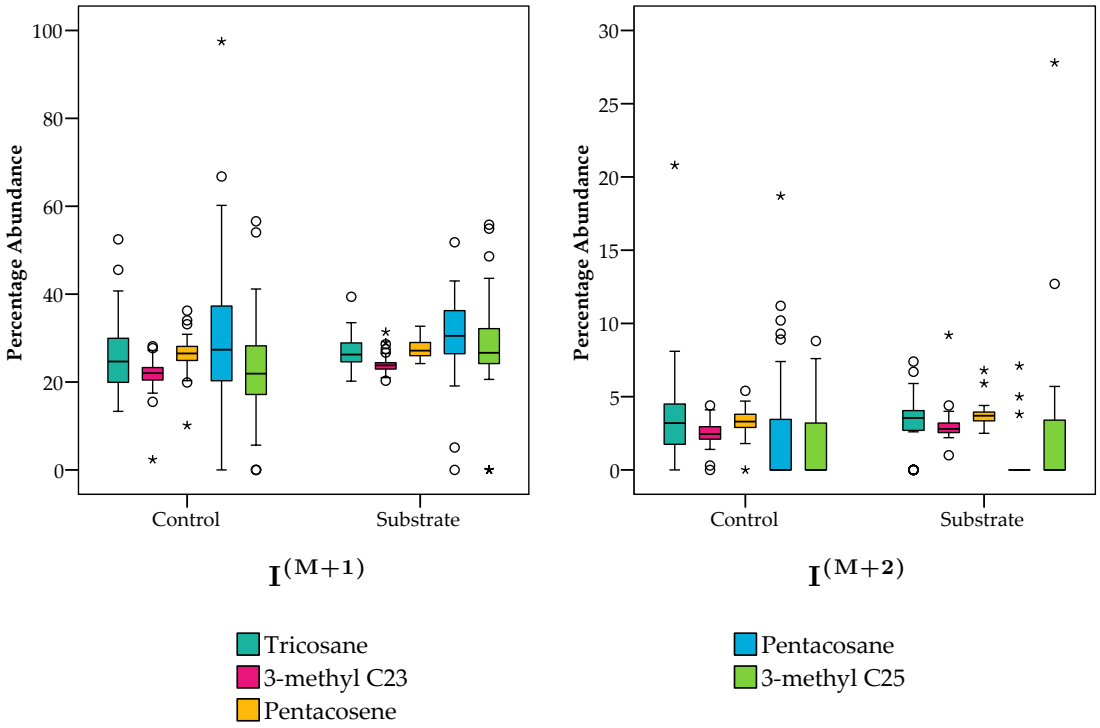


Figure 4.25: Boxplots showing the data for *Myrmica scabrinodis* and the substrate propionic-2,2-d_2 acid-d; $I^{(M+1)}$ on the left, and $I^{(M+2)}$ on the right.

Figure 4.25 shows that, again, because the group sizes are so big, there are many outliers and extreme outliers. Looking at the data presented there does not appear to be any clear difference between the groups for either the M+1 and M+2 data. To confirm this Table 4.8 shows the results of the Mann-Whitney U-tests.

The p values shown in Table 4.8 indicate that there is some statistical significance within this data set. For both $I^{(M+1)}$ and $I^{(M+2)}$ for 3-methyltricosane p=0.000, indicating clear significance. There is also significance indicated for 3-methylpentacosane, but only for $I^{(M+1)}$ (p=0.005). In order to say that this is a clear incorporation of the substrate during the biosynthesis of methyl-branched compounds it would be necessary to also have a statistically significant result for $I^{(M+2)}$, however this is not the case. Despite this, these results do suggest that the initial result seen for *Myrmica sabuleti* is also repeated here, the high p value for $I^{(M+2)}$ and 3-methyl C25 most likely due to

Table 4.8: The probability values from the Mann-Whitney U-tests for *Myrmica scabrinodis*, for propionic-2,2-d_2 acid-d, and both isotope peaks.

Compound	p value	
	Propionic-2,2-d_2 acid-d	
	$I^{(M+1)}$	$I^{(M+2)}$
Tricosane	0.047	0.276
3-methyl C23	0.000	0.000
Pentacosene	0.038	0.034
Pentacosane	0.181	0.000
3-methyl C25	0.005	0.298

poor quality data. For both species however there are a few other significant results for straight chain hydrocarbons, the reasons for this will be explored later in the discussion section.

4.3.3.3 Discussion

The results of the substrate experiment with propionic-2,2-d_2 acid-d and *Myrmica sabuleti* showed that the previously obtained result from the preliminary propionate experiment was not observed again. The data from *Myrmica scabrinodis* suggested that the substrate was used during the biosynthesis of methyl-branched hydrocarbons but again it was not as clear a result as that seen previously (albeit for a different species). Together these results were somewhat surprising but not completely unexpected. The ants that are used for these experiments are collected from the wild, therefore they are of unknown age, status and health. When this experiment was carried out previously the ants had been collected and maintained within the lab for a few months prior. In contrast, for the expansion of this experiment a greater number of ants were required and so extra colonies were collected especially and used immediately for this experiment. Ants that are kept within the lab are fed on an artificial diet; whilst this provides nutritional requirements, it does not replicate what would be naturally foraged in the wild. Therefore the reason that the somewhat contradicting results were observed

could be down to the natural stores of biologically useful molecules that are obtained from a varied diet. The ants used initially for the preliminary experiments could have been effectively starved of these natural resources due to being kept in the lab for a few months and therefore readily utilised the labelled propionate molecules. In contrast the recently obtained wild ants might have had plenty of reserves and therefore had less need for the labelled propionate molecules. This is purely a suggested theory and in order to prove whether this is the case further experimentation would be required, however the effect of 'lab age' on the biosynthesis of cuticular hydrocarbons is deemed to be outside the scope of this thesis.

Alternatively the difference in results could be down to the quality of the initial data, see Appendix B. The accuracy of the ion count measurements depends on the amount of measured hydrocarbon. This can vary due to many reasons; sensitivity of the instrument for example, or the physical amount of hydrocarbon present. When the experiment was initially run, the average ion count for M across the substrate samples was 77,000 counts. When this experiment was repeated, the comparable average count was 17,000 counts. As mentioned this could be due to the status of the instrument; a dirty ion source for example can cause low sensitivity [14]. A lack of sensitivity causes lower ion counts to be recorded which has a significant impact on the analysis of the results. Appendices B.35-B.36 show the raw data for *Myrmica scabrinodis* and for propionic-2,2-d_2 acid-d. In particular, the lack of ion count data collected for 3-methyl C25 at the M+2 level is evident. This leads to many values of 0.0%, and although there are values that are very much larger than those expected, namely for samples 'Sub 19 'and 'Sub 49', the prevalance of values that are 0.0% means that statistical significance is not found, even though the appearance of the raw data contradicts this.

As well as instrumental issues the change in results could also be due to the physical amount of hydrocarbons present on the ant's cuticle. There are several studies that investigate the relationship between environmental factors and hydrocarbon produc-

tion in ant and non-ant species [15–17]. In a study by Wagner et al. [16] the authors investigated the difference in CHC composition between forager and non-forager *Pogonomyrmex barbatus* ants. The results of this study showed that foragers and patrollers had CHC profiles with a higher proportion of *n*-alkanes compared to the non-foraging maintenance workers. Further investigation related these differences to temperature and humidity, with warm dry conditions triggering these changes.

This is an interesting theory; often the first ants selected for the experiments performed within this project are those that are the most easily accessible, i.e. the ones that are on the surface of the nest fragment, likely the foragers. As the foragers are removed the nest fragment is broken up and ants from further within the fragment are selected. It is therefore entirely possible that some boxes are made up of predominantly forager ants, whilst others are predominantly non-forager ants. If the amount of hydrocarbon is dependant on worker role then this biased collection process could lead to some skew within the amounts of hydrocarbons within some boxes and hence the quality of the results. Having said this, the study mentioned above found that the cause of the difference in hydrocarbon amounts was driven by the differing temperature and humidity that forager and non-forger ants are exposed to. This is likely less of an effect for the lab ants as the conditions are more controlled, even foraging ants are still not exposed to extreme temperatures or humidities. The large variation is therefore more likely to be due to instrumental discrepancies caused by changes in sensitivity.

It is worth however considering the effects of changes in relative humidity. For the repeat experiment the containers were modified by adding a thin layer of plaster of paris to the base. This was done to increase the humidity level within the box to avoid desiccation and decrease the mortality rate during the experiment. It is possible that the increased humidity led to a reduction in the amount of hydrocarbons produced simply because in such a humid environment the risk of desiccation was reduced. However as previously mentioned one of the main issues with using ants from the wild

is that they are totally unknown in terms of their age, status, health and role. It is therefore possible that there are other factors which are influential and which cannot be controlled.

Despite the fact that the initial result was not found to be repeatable it does not mean that it was erroneous. The quality of the raw data was very high with the individual samples showing high measured levels of hydrocarbons, this ensures the accuracy of the ion count measurements. In addition the p values from the first of the repeats were very statistically significant, these were not p values that were near the threshold for significance, therefore a high amount of confidence can be placed on this first result, even if a repeat did not yield the same outcome.

There was also incorporation seen for some of the alkenes and alkanes. As previously mentioned ants can use propionate molecules to metabolise into acetates, these can then be used for lengthening the straight chain part of a hydrocarbon. It could be that this process is causing the incorporation observed for some of the alkenes and alkanes. Rather than the propionates being used during the incorporation of the methyl-branch they are instead metabolised to form acetates according to the scheme shown in Figure 4.26. As seen from this mechanism, when the labelled propionate used in this experiment is metabolised to form acetate, one deuterium label remains within the acetate. This has the potential to then be incorporated into a straight chain hydrocarbon. As suggested, this is likely to be a more minor biosynthetic route and because it contains a lot of steps, successful incorporation is less likely, which may explain why sometimes only $I^{(M+1)}$ is significant, and the seemingly random nature of the incorporation.

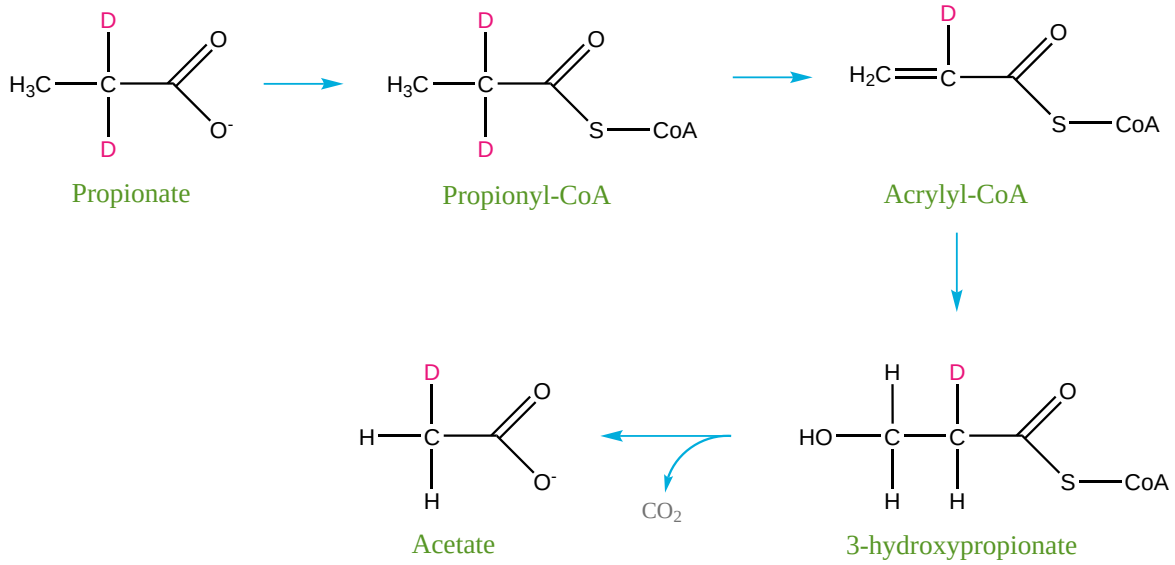


Figure 4.26: Schematic showing the hypothesised metabolism of propionate into acetate, in this scheme the deuterium labels are indicated. Adapted from [13].

4.4 Conclusions

This first chapter focussed on three initial experiments. These were very much preliminary experiments, carried out in order to test the experimental design, species suitability, and chemical and statistical analysis methods. However given that these were fairly basic in format, some interesting results and insights were gained. Firstly it was appreciated that not all species were suited to use in this kind of work, instead species should be chosen which show a clear chemical profile with abundant amounts of cuticular hydrocarbon present to aid in analysis. The experimental design proved to be fairly successful, although some modifications were made to the ant's environment as well as increasing the number of samples and adding extra controls and repeats. These changes were all aimed at improving the success and rigorousness of the experiments.

The first of the experiments used simple acetate molecules; it was found that both *Formica lemani* and *Myrmica sabuleti* incorporate acetate molecules into their alkene, alkane and monomethyl alkane compounds. However the extent of this incorporation appears to depend on the individual cuticular compounds, as well as the substrate and species. During this experiment two different substrates were tested. Previous research suggested that the sodium [1-^{13}C]acetate would label the even carbons and the [2-^{13}C]acetate the odd [3]. On this basis it was expected that the results of the experiment using sodium [2-^{13}C]acetate would show greater incorporation as there are more odd numbered carbons within our species. Although this trend was observed, these experiments were not performed simultaneously so it can be suggested that it is impossible to make direct comparisons between them. They also utilised different groups of ants which are not genetically controlled and may have been of differing status, age and health. However, both of these substrates showed clear incorporation into the biosynthesised hydrocarbons and as such, sodium [1-^{13}C]acetate was later used as a positive control to determine whether the ants were successfully feeding, and to ensure that there was no contamination of the food which might cause increased mortality

during the experiment.

Initial research also suggested that propionates are biosynthetically important molecules and are used during the biosynthesis of methyl-branches. Therefore two simple propionate molecules were tested. Due to the commercial availability of these molecules it was not possible to get [2-^{13}C]propionate as it was too expensive to obtain the required amount. Instead propionic-2,2-d_2 acid-d was used, although not ideal, it still allowed a greater insight into the biosynthetic mechanism. The results for this experiment showed that there was a clear difference in incorporation depending on the species. *Formica lemani* only incorporated the [1-^{13}C]propionate, whilst *Myrmica sabuleti* showed clear incorporation of both substrates. Both species showed a strong tendency to use the substrates for the biosynthesis of the methyl-branched compounds, although there was also statistical difference observed for some of the straight chain molecules. This was attributed to the insects ability to metabolise propionate to acetate. The resulting acetate molecules can then be used during the biosynthesis of straight chain hydrocarbons.

The results of the propionic-2,2-d_2 acid-d experiment for *M. sabuleti* were particularly interesting as research of the literature suggested that if the assumed mechanism was used then the labels from the propionic-2,2-d_2 acid-d would be lost during the modification and incorporation of this compound. Therefore it was surprising to see this so clearly incorporated into the methyl-branched hydrocarbons. This suggested that the previous mechanistic pathways described may be slightly different for this species and suggested that further research was necessary to determine whether this was the case. However this finding did reinforce an important point, that even though prior research suggested mechanistic pathways for the biosynthesis of hydrocarbons, these have not been proven for all species and that there could also be the potential for insects to be able to use minor, previously unknown biosynthetic pathways. This highlights the importance of not assuming a particular outcome, even if it is a fundamental aspect which is being tested.

As a result of this the experiment was expanded and repeated, with the addition of another *Myrmica* species; *Myrmica scabrinodis*. The results from this experiment were disappointing. There was no clear incorporation, although some statistical differences were seen for some of the groups for some of the compounds. Whilst it would have been beneficial to have repeated this work again, it was instead decided to try different substrates. Unfortunately, as previously mentioned, the main issue with this work is that, unlike working with fruit flies or cockroaches, as much of the previous experimentation has, the genetics of these ants are not controlled. These are wild populations, with many variables that cannot be removed. These variables may have no effect on the way the substrates are incorporated, or could be hugely important. This concept in itself would be an entirely separate area of research outside the scope of this thesis.

References

[1] Gu X, Quilici D, Juarez P, Blomquist GJ, and Schal C, 'Biosynthesis of hydrocarbons and contact sex pheromone and their transport by Lipophorin in females of the German cockroach *Blattella germanica*,' (1995), *J. Insect Physiol.*, 41(3), 257-267

[2] Chase J, Jurenka RA, Schal C, Halarnka PP, and Blomquist GJ, 'Biosynthesis of methyl-branched hydrocarbons of the German cockroach *Blattella germanica* (L.) (Orthoptera Blattellidae),' (1990), *Insect Biochem.*, 20(2), 149-156

[3] Dillwith JW, Nelson JH, Pomonis JG, Nelson DR, and Blomquist GJ, 'A ^{13}C NMR study of methyl-branched hydrocarbon biosynthesis in the housefly,' (1982), *J. Biol. Chem.*, 257(19), 11305-11314

[4] Soroker V, Vienne C, and Hefetz A, 'Hydrocarbon dynamics within and between nestmates in *Cataglyphis niger* (Hymenoptera: Formicidae),' (1995), *J. Chem. Ecol.*, 21(3), 365-378

[5] Bestmann HJ, Übler A, and Hölldobler B, 'First biosynthetic studies on trail pheromones in ants,' (1997), *Angew. Chem. Int. Ed. Engl.*, 36(4), 395-397

[6] Morgan ED, *Biosynthesis in Insects*, (Royal Society of Chemistry, 2010)

[7] Soroker V, and Hefetz A, (2000) *J. Insect Physiol.*, 46(7), 1097-1102

[8] Blomquist GJ, *Insect Hydrocarbons*, (Cambridge University Press, 2010)

[9] Gullan PJ, and Cranston PS, *The Insects: An Outline of Entomology*, (Chapman and Hall, 1994)

[10] Martin SJ, and Drijfhout FP, 'Nestmate and task cues are influenced and encoded differently within ant cuticular hydrocarbon profiles,' (2009), *J. Chem. Ecol.*, 35(3), 368-74

[11] Lockey KH, 'Lipids of the insect cuticle: Origin, composition and function,' (1988), *Comp. Biochem. Physiol.*, 89B(4), 595-645

[12] Soroker V, Lucas C, Simon T, Fresneau D, Durand JL, and Hefetz A, 'Hydrocarbon distribution and colony odour homogenisation in *Pachycondyla apicalis*,' (2003) *Insectes Sociaux*, 50(3), 212-217

[13] Halarnkar PP, and Blomquist GJ, 'Comparative aspects of propionate metabolism,' (1989), *Comp. Biochem. Physiol.*, 92B(2), 227-231

[14] Agilent Technologies, *5975 Series MSD Troubleshooting and Maintenance Manual, 4th Edition*, (Agilent Technologies, 2012)

[15] Martin SJ, Shemilt S, and Trontti K, 'Nest-mate recognition cues are not used during or influenced by mating in the ant *Formica exsecta*,' (2013), *Ethol. Ecol. Evol.*, 26, 40-48

[16] Wagner D, Tissot M, and Gordon DM, 'Task-related environment alters the cuticular hydrocarbon composition of harvester ants,' (2001), *J. Chem. Ecol.*, 27, 1805-1819

[17] Frentiu FD, and Chenoweth SF, 'Clines in cuticular hydrocarbons in two *Drosophila* species with independent population histories,' (2010), *Evolution*, 64, 1784-1794

Chapter 5

Labelled substrate feeding experiment: amino acids

5.1 Introduction

In terms of the biosynthesis of cuticular hydrocarbons there are many biologically important molecules which can be used in the process, either as precursors or modifiers. Included in this group are acetates and propionates, which are discussed in Chapter 4. Another important group of compounds are the amino acids. Amino acids are essential biological molecules and act as building blocks for the formation of proteins [1]. In terms of the biosynthesis of cuticular hydrocarbons in insect species, amino acids are used during the biosynthesis of monomethyl alkanes, but only when the branch is in the 2-methyl position. In this way the amino acids are used as precursors to the synthesis, inserting the methyl group in the 2 position and providing a carbon chain that can be further extended [2]. This occurs in the same way as that described previously; acetate, in the form of malonate, is used to elongate the carbon chain until the required length is reached. Specifically with this biosynthetic route, only the amino acids valine and leucine are used. Valine is used to biosynthesise 2-methyl hydrocarbons with an even chain length, whilst leucine is used for 2-methyls containing an odd chain length. However cuticular hydrocarbon profiles containing methyl branches in the 2 position are very uncommon; methyl-branched compounds where the methyl group is located on an odd numbered carbon are far more abundant, as is the case with our study species [3].

In addition to being used as precursors for 2-methyl compounds, some amino acids can also be metabolised to form propionates and hence can be used during the biosynthesis of internally branched hydrocarbons. This pathway is generally used by species which lack, or have low levels of vitamin B_{12} [4]. The amino acids which are suitable for this metabolism are limited to valine, isoleucine and methionine. However all of these can be metabolised to form propionates, and as previously described, this propionate can then be converted to methylmalonate and used to introduce the internal methyl branch. Based on the knowledge that amino acids are biologically important molecules it was decided to investigate them as substrates within a feeding experiment.

One of the main issues throughout this project has been the commercial availability of suitable substrates with isotopic labels. There is only a fairly small range of suitable substrates with the labels in the correct locations, and some are prohibitively expensive. Based on the availability of suitable substrates two amino acids were selected; valine was chosen because research suggested that incorporation would be seen and leucine was chosen because the literature suggested that incorporation would not be observed [2]. Whilst it may seem a strange choice to choose a substrate that research suggested would not be incorporated, the results of the previous chapter reinforced that because these species had never been tested it was important not to make assumptions about these species, and their biosynthetic pathways. Therefore the chosen amino acids were valine and leucine.

Aims & Objectives

The aims of this chapter are twofold. The first aim is to determine if leucine is incorporated into the cuticular hydrocarbons of the studied species. Analysis of the literature suggests that this substrate would not be incorporated and therefore it is important to see if this is the case, and if it is not, what the possible minor mechanisms behind the incorporation could be. It is also important to see if there are any differences between our two study species. The second aim was to test whether the assumed metabolic

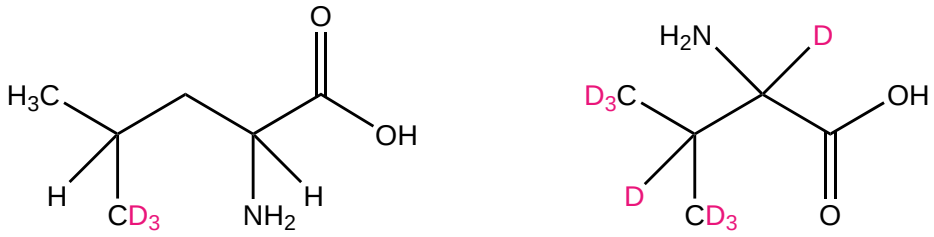
breakdown of valine to form propionate leads to incorporation being seen for just the methyl-branched hydrocarbons, as the literature suggests, and if so, is this observed for both species or just one. Additionally, if incorporation is observed, any differences between the two species will be analysed and possible reasons suggested.

5.2 Experimental

The experiment involving labelled amino acids consisted of two distinct experiments, with different substrates used in each. This experimental section will deal with each part separately.

5.2.1 Deuterated substrates

The first of the two experiments utilised deuterated substrates. For this experiment two species of ants were used; *Formica lemani* and *Myrmica sabuleti*. The two deuterated substrates used were DL-valine d_8 (98% purity) and L-leucine 5,5,5-d_3 (99%), both purchased from Cambridge Isotope Laboratories Inc. Both of these amino acids are labelled with multiple deuterium atoms as shown by the chemical structures in Figure 5.1.



L-Leucine 5,5,5-d_3 DL-Valine d_8

Figure 5.1: The structure of the substrates used for the first part of the amino acid substrate experiment, the position of the deuterium labels can be clearly seen.

For this experiment, eight plastic boxes, (two substrate and two controls per species), measuring 17.0 x 11.5 x 4.5 cm were prepared by painting the sides with Fluon anti-stick coating (Blades Biological Ltd.) and the bottom with plaster of paris and a small amount of soil. For each species and substrate a control and substrate box was set up. Each box had approximately 25 worker ants of either of the two species added.

Modified diets were prepared using the standard diet (see Section 3.1). To 50 g of this

diet, 1 g of L-leucine or DL-valine (Sigma Aldrich) (2% w/w) was added, which served as a control. Each substrate diet was made by adding either 100 mg of DL-valine d_8 or L-leucine 5,5,5-d_3 (Cambridge Isotope Laboratories Inc.) to 5 g of standard diet to give a resulting concentration of 2 % w/w. All diets were cut into small cubes (approximately 0.5 cm^3). One cube was placed on to a small piece of plastic inside each box and the food was replaced every other day. All diets were stored in the freezer and defrosted prior to use and replaced every other day. The experiment was stopped after 31 days.

After 31 days all remaining ants from each of the eight boxes were individually extracted and analysed, according to the methods described in Sections 3.2.1 and 3.2.2. For each species the chemical profile was studied and eight key compounds were selected. The majority of the compounds chosen were the most abundant for each species, however they also represented the different compound types present. The most abundant compounds were chosen because the greater the amount of a particular compound present on the cuticle the higher the associated ion count from the MS detector, and thus the greater the accuracy of the measurement. Note that for the methyl-branched compounds, suitable fragments were chosen because methyl-branched compounds rarely exhibit the molecular ion in the mass spectrum due to fragmentation. Given that both of these substrates contained multiple deuterium labels it was decided to analyse the $I^{(M+3)}$ data as well as the $I^{(M+1)}$ and $I^{(M+2)}$; it was hoped that this might reveal any trends that may otherwise remain hidden.

5.2.2 ^{13}Carbon substrates

For the second part of this experiment slightly different substrates were used. Again leucine and valine were used but this time the isotopic labels were based on carbon and nitrogen atoms. The choice to repeat this experiment with slightly different substrates was taken in the hope that because these isotopic atoms are less labile than deuterium

atoms they may provide clearer insight into the biosynthetic processes. The substrates used in this part of the experiment were L-leucine $^{13}C_6$,^{15}N and L-valine $^{13}C_5$,^{15}N, both purchased from Cambridge Isotope Laboratories Inc. (99% purity). The structure of these labelled substrates is shown in Figure 5.2.

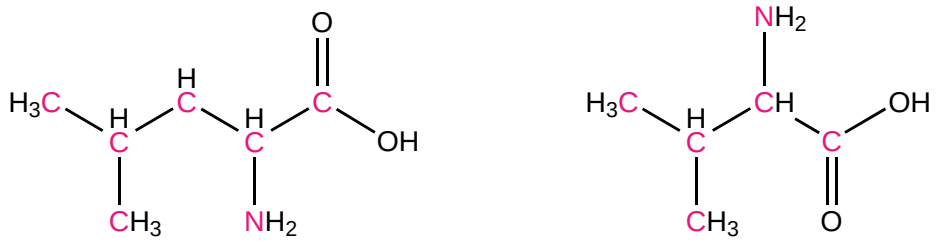


Figure 5.2: The structure of the substrates used for the second part of the amino acid substrate experiment, the position of the labelled atoms are shown in pink.

For this experiment ten plastic boxes measuring 17.0 x 11.5 x 4.5 cm were prepared by lining the sides with Fluon anti-stick coating (Blades Biological Ltd.) and the bottom with a small amount of soil. For each species and substrate a control and substrate box was set up. There was also an additional control used for each species, see below for details of this. The number of ants used for each box is shown in Figure 5.3. Note that due to the availabilty of *Myrmica sabuleti* ants, the sodium [1-^{13}C]acetate control contained fewer individuals.

In the same way as before modified diets were prepared using the standard diet (see Section 3.1). To 50 g of this diet, 1 g of L-leucine or DL-valine (Sigma Aldrich) (2% w/w) was added, which served as a control. Each substrate diet was made by adding either 250 mg L-valine $^{13}C_5$,^{15}N or 100 mg of L-leucine $^{13}C_6$,^{15}N to either 12.5 g or 5 g respectively of standard diet to give a resulting concentration of 2% w/w. In addition to the four diets described above there was a fifth prepared; this contained 2% w/w sodium [1-^{13}C]acetate. This was included to determine whether the ants were successfully feeding, as previous results had indicated that *F. lemani* did not demonstrate as

Figure 5.3: The setup used for the second amino acid substrate experiment. The different colours indicate the different substrates. The numbers in the box refer to the number of ants used per box.

prolific incorporation as other species and therefore may have a possible lower food intake than *M. sabuleti*. All diets were cut into small cubes (approximately 0.5 cm^3). One cube was placed on to a small piece of plastic inside each box and the food was replaced every other day. All diets were stored in the freezer and defrosted prior to use each time. The experiment was stopped after 29 days. Figure 5.3 summarises the experiment (the number of ants per box is also shown).

5.3 Results & Discussion

The data obtained from these experiments exhibited large amounts of variance therefore Mann-Whitney U-tests were performed on each data set to establish its significance compared to the relevant control. To visually show the data, box plots were also created, each showing the median and range of the data. However, whilst a visual representation of the data, these box plots do not confirm significance. For this experiment the results for each amino acid and each substrate will be presented separately.

5.3.1 Leucine

5.3.1.1 L-leucine 5,5,5-d_3

Formica lemani

The boxplots shown in Figure 5.4 are an effective way of showing the results from the first of the amino acid experiments. From Figure 5.4 it can be seen that the values for $I^{(M+3)}$ data are very low, with a few outliers for most of the compounds. The data for $I^{(M+1)}$ and $I^{(M+2)}$ is much more meaningful, however within these sets there is a lot of variation, particularly for the $I^{(M+2)}$ control data.

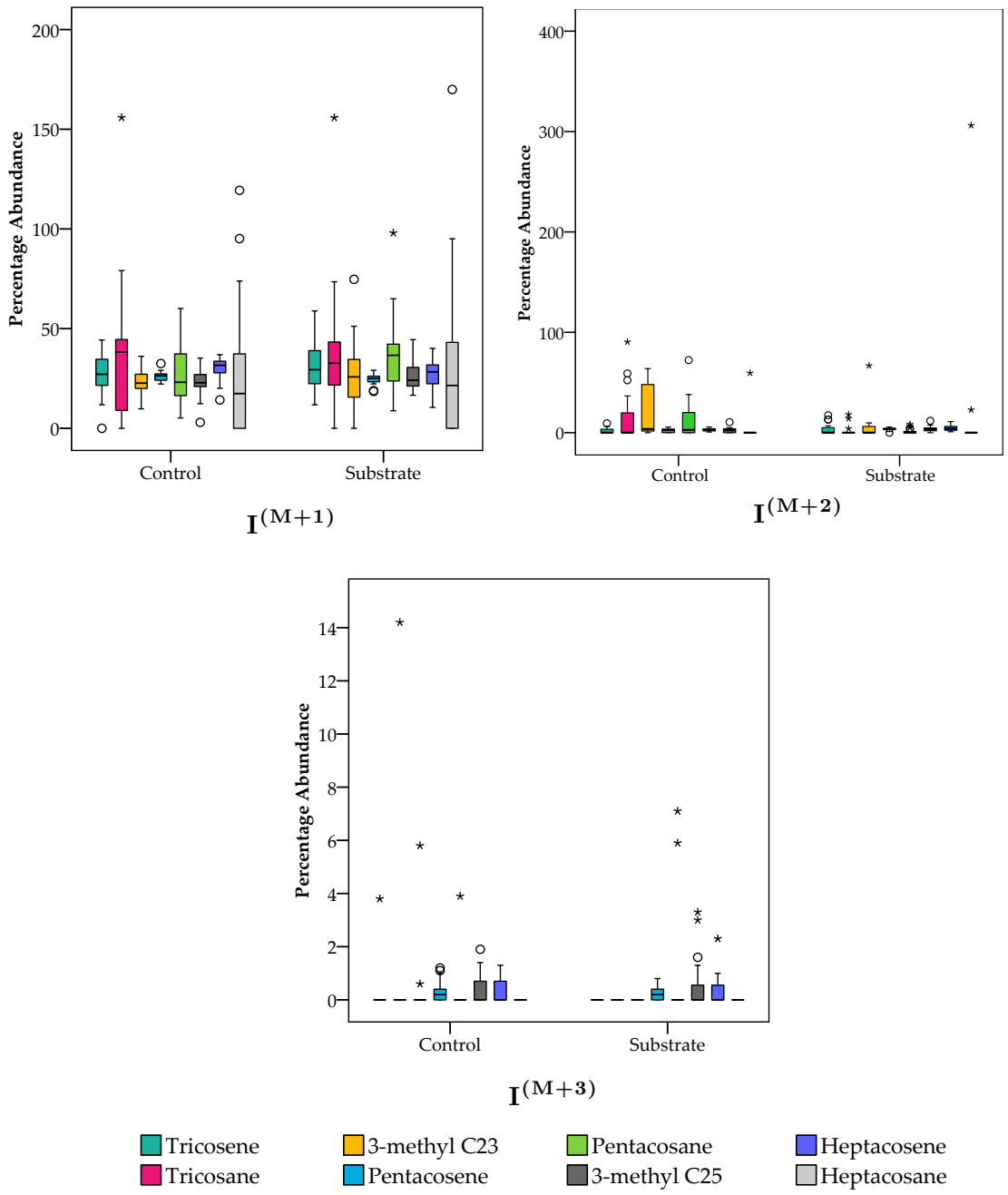


Figure 5.4: Boxplots showing the data for *Formica lemani* for L-leucine 5,5,5-d_3. $I^{(M+1)}$ is on the left, $I^{(M+2)}$ on the right and $I^{(M+3)}$ below.

In order to determine statistical significance Mann-Whitney U-tests were performed, the results of which can be seen in Table 5.1. The Mann-Whitney U-test compares the abundance values for each substrate compared to the relevant control for each isotope peak i.e $I^{(M+1)}$, $I^{(M+2)}$ etc. Note that for this set of results, n=24 for the leucine control and 25 for the labelled leucine substrate.

Table 5.1: The probability values from the calculated Mann-Whitney U-tests for *Formica lemani*, for L-leucine 5,5,5-d_3, and all three isotope peaks. Note that any p values less than 0.050, and therefore significant, are underlined for ease of reference. Any p values less than 0.050 which are italicised may be disregarded and are due to the median rank of the control being greater than that of the substrate group.

Compound	p value L-leucine 5,5,5-d_3		
	$I^{(M+1)}$	$I^{(M+2)}$	$I^{(M+3)}$
Tricosene	0.264	0.360	0.490
Tricosane	0.439	*0.010*	0.490
3-methyl C23	0.377	*0.010*	0.235
Pentacosene	0.159	0.001	0.394
Pentacosane	0.090	*0.008*	0.255
3-methyl C25	0.284	0.232	0.186
Heptacosene	0.069	0.004	0.247
Heptacosane	0.305	0.358	1.000

The results of the Mann-Whitney U-tests indicate that any statistical difference between the two groups can only be seen when studying the probabilities for the $I^{(M+2)}$ data, that is the isotope peak that is two mass units (2 Da) greater than the molecular mass peak. In the table any p values less than 0.050 which are statistically significant are underlined. It can be seen that this significance is only seen for the unsaturated straight chained compounds pentacosene and heptacosene.

The significant results are seen only at the $I^{(M+2)}$ level. This may indicate that for every labelled substrate molecule incorporated, only two deuterium labels make it through intact to the final hydrocarbon. This may suggest that when this substrate is metabolised by the ants one of these labels is lost.

Myrmica sabuleti

The following data refers to the data obtained for *Myrmica sabuleti* for the L-leucine 5,5,5-d_3 substrate. For this experiment there were just seven control samples, however there were 18 substrate samples.

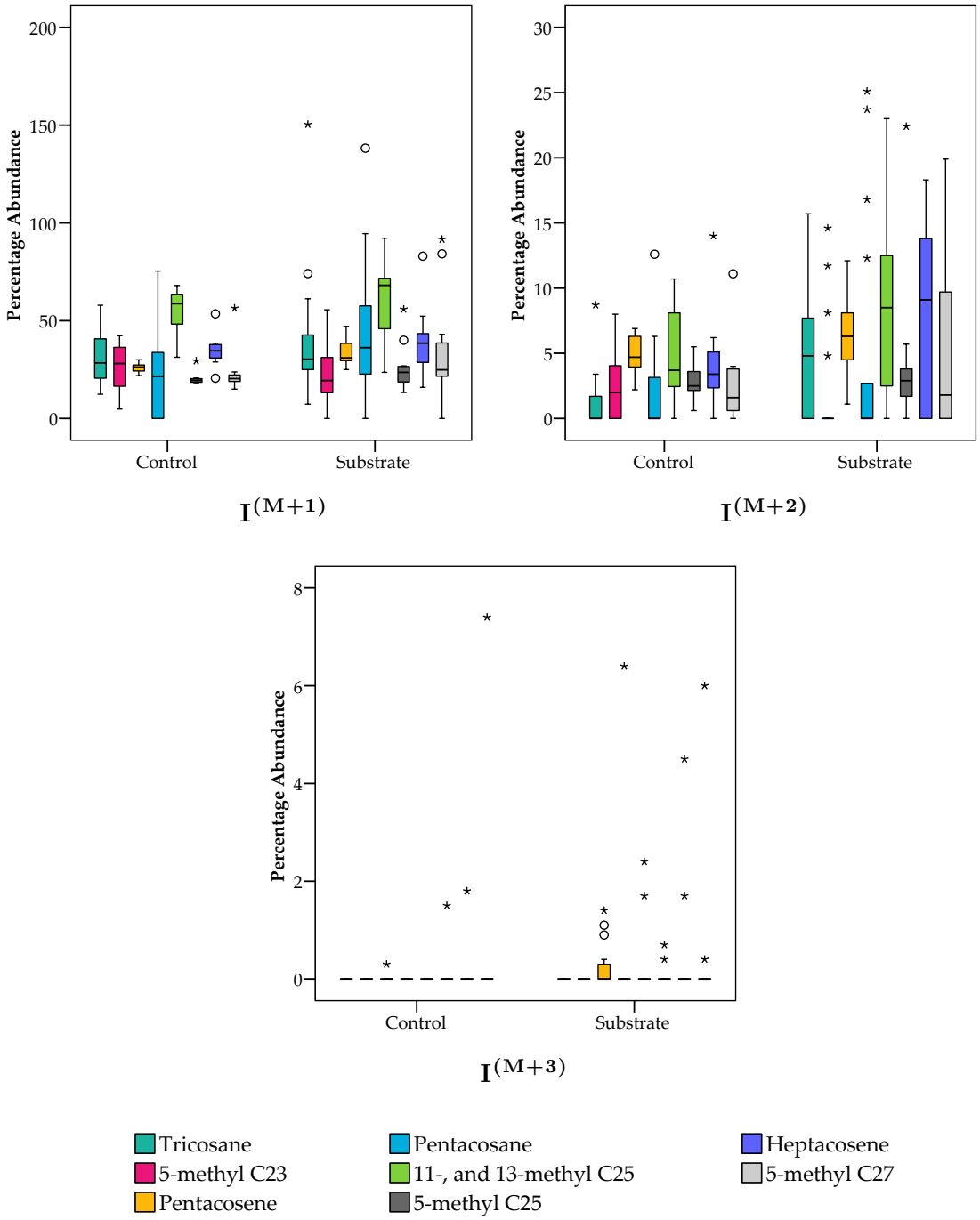


Figure 5.5: Boxplots showing the data for *Myrmica sabuleti* for L-leucine 5,5,5-d_3. $I^{(M+1)}$ is on the left, $I^{(M+2)}$ on the right and $I^{(M+3)}$ below.

As these boxplots show, again there is very little meaningful data for $I^{(M+3)}$; the raw data sheets show that the majority of the values are 0.0%, with a few others which are indicated in the plot as outliers (circles) and extreme outliers (asterisks). See Appendices C.7-C.8 for the data sheets.

For the $I^{(M+1)}$ there does not appear to be any clear difference, except for pentacosane where the median percentage abundance for the substrate is greater than that for the control. There are more differences apparent in the data for $I^{(M+2)}$, with the median values for several compounds appearing to be greater for the substrate than for the control. In order to analyse the difference in the groups in more detail, Mann-Whitney U-tests were performed, the results of which are shown in Table 5.2.

Table 5.2: The probability values from the calculated Mann-Whitney U-tests for *Myrmica sabuleti*, for L-leucine 5,5,5-d_3, and all three isotope peaks. Note that any p values less than 0.050, and therefore significant, are underlined for ease of reference.

Compound	p value L-leucine 5,5,5-d_3		
	$I^{(M+1)}$	$I^{(M+2)}$	$I^{(M+3)}$
Tricosane	0.287	0.060	1.000
5-methyl C23	0.378	0.241	1.000
Pentacosene	<u>0.000</u>	0.113	0.143
Pentacosane	0.109	0.443	0.720
11-, and 13-methyl C25	0.120	0.118	0.355
5-methyl C25	0.152	0.494	0.335
Heptacosene	0.182	0.111	0.490
5-methyl C27	0.102	0.426	0.350

The results of the Mann-Whitney U-tests indicate that despite the appearance of the box plots there is only one result which is statistically significant; that of pentacosene for the $I^{(M+1)}$ data. The lack of significance shown by the U-tests is likely down to the very small number of control samples for this data set (n=7). In order to perform the U-test the software first ranks all the results, this removes the variation. The small-

est value is assigned a rank of 1 and so on, therefore large differences in the median ranks of the group provide an indication of the difference in values between datasets. However the box plots do not use the median rank, instead they plot the data in terms of percentage abundance, with the median on the box plot representing the median percentage abundance value, rather than the median rank. This means that the box plots are highly influenced by varied data, whilst Mann-Whitney removes this bias. Within the control samples there is a lot of variance, therefore a greater number of samples would have reduced the influence of this effect.

5.3.1.2 L-leucine $^{13}C_6$,^{15}N

Formica lemani

Immediately upon looking at the mass spectra data, it was apparent that incorporation had occurred for this species and substrate. In addition the data indicated that incorporation of the substrate was widespread with a large number of extra isotopes present. See Figure 5.6 for an example full mass spectrum for *Formica lemani* and Figure 5.7 for the comparative mass spectrum extract.

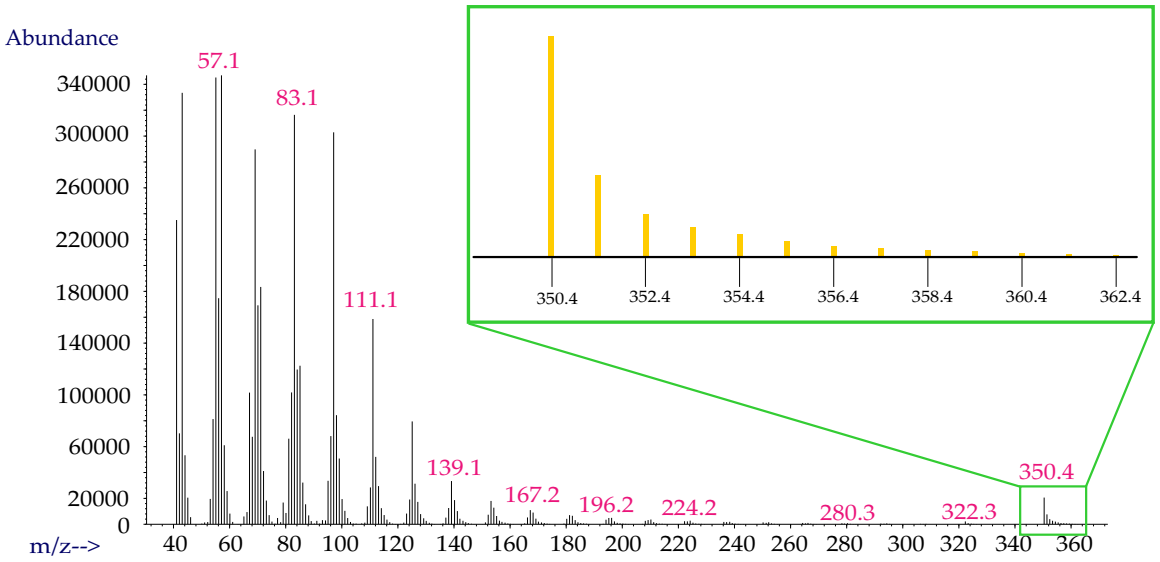


Figure 5.6: Example mass spectrum for *Formica lemani* and the L-leucine $^{13}C_6$,^{15}N substrate experiment. Large amounts of incorporation at various M+n levels, represented by yellow bars, can be clearly seen indicating widespread incorporation.

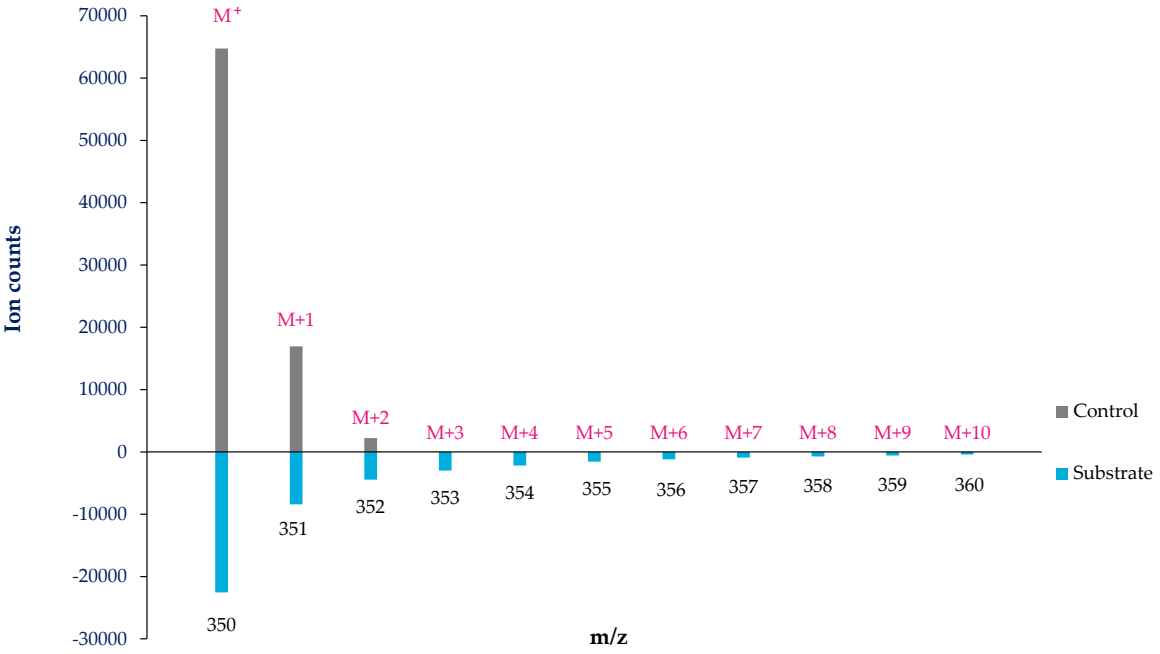


Figure 5.7: Extracted mass spectrum for *Formica lemani* and the L-leucine $^{13}C_6$,^{15}N substrate experiment showing average comparative ion counts for control and substrate data sets for the compound pentacosene.

Due to this widespread incorporation, the procedure for the data analysis for this species and substrate was adjusted and the values for $I^{(M+X)}$ were recorded up to, and including $I^{(M+10)}$. This was so that any trends within the data could be explored. However for the purposes of comparing this data against the control, only the peaks for $I^{(M+X)}$, where X=1-3, will be initially presented so that the data for the substrate and control is directly comparable.

It should also be noted that because this was a different colony to that used before the abundances of the compounds present within the chemical profile were slightly different. This meant that slightly different compounds were selected for analysis.

The following boxplots show the results of the L-leucine $^{13}C_6$,^{15}N substrate for $I^{(M+1)}$, $I^{(M+2)}$ and $I^{(M+3)}$.

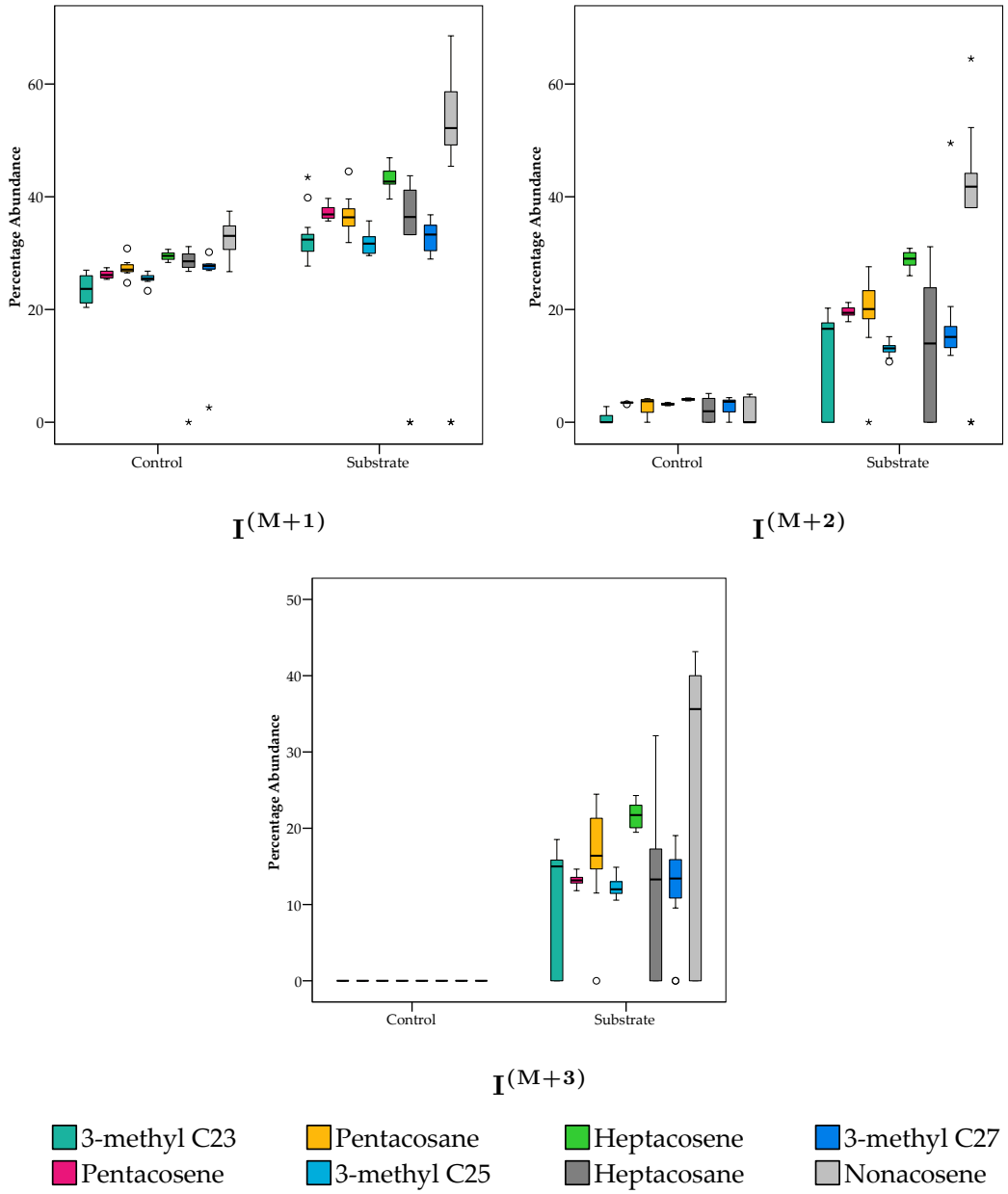


Figure 5.8: Boxplots showing the data for *Formica lemani* for L-leucine $^{13}C_6$,^{15}N. $I^{(M+1)}$ is on the left, $I^{(M+2)}$ on the right and $I^{(M+3)}$ below.

Figure 5.8 shows the range and variability of the data. From these boxplots it can be seen that the range of the substrate data is greatest for $I^{(M+3)}$. In contrast the data range is narrowest for $I^{(M+1)}$. There does not appear to be any correlation between compound type and the range of the data as the biggest range is seen for nonacosene (pale grey), heptacosane (dark grey) and 3-methyl C23 (3-methyltricosane), shown in turquoise; these are three different compound types. This variation is also not caused by the abundances of the compounds, as although these are the less abundant com-

pounds, 3-methyl C27 is also not present in large amounts and therefore on this basis it might be expected that this compound has a large variation but this is not the case. The large variation seen here is due to several of the samples for these compounds having percentage abundance values of 0.0%, whilst some of the values are much higher. This could be due to the sensitivity of the instrument or some other unknown effect.

Table 5.3 shows the Mann-Whitney U-test data for *Formica lemani* and L-leucine $^{13}C_6$,^{15}N.

Table 5.3: The probability values from the calculated Mann-Whitney U-tests for *Formica lemani*, for L-leucine $^{13}C_6$,^{15}N, and all three isotope peaks. Note that any p values less than 0.050, and therefore significant, are underlined for ease of reference.

Compound	p value L-leucine $^{13}C_6$,^{15}N		
	$I^{(M+1)}$	$I^{(M+2)}$	$I^{(M+3)}$
3-methyl C23	<u>0.000</u>	<u>0.003</u>	<u>0.004</u>
Pentacosene	<u>0.000</u>	<u>0.000</u>	<u>0.000</u>
Pentacosane	<u>0.000</u>	<u>0.000</u>	<u>0.000</u>
3-methyl C25	<u>0.000</u>	<u>0.000</u>	<u>0.000</u>
Heptacosene	<u>0.000</u>	<u>0.000</u>	<u>0.000</u>
Heptacosane	<u>0.010</u>	0.077	<u>0.009</u>
3-methyl C27	<u>0.000</u>	<u>0.000</u>	<u>0.000</u>
Nonacosene	<u>0.002</u>	<u>0.002</u>	<u>0.002</u>

The data shows that with the exception of heptacosane for $I^{(M+2)}$ all the probability values are less that 0.050 and therefore significant. This is not an unexpected result as the mass spectra themselves showed clear incorporation. However although this data is certainly interesting it does not show clearly the extent of the incorporation. As previously mentioned the data for this substrate, and this species, was recorded up to, and including $I^{(M+10)}$. This was because after $I^{(M+10)}$ the percentage abundances were approaching zero and therefore it was felt that up to $I^{(M+10)}$ was an appropriate measurement. The graphs shown in Figure 5.9 represent the varying percentage abun-

dances of the ion counts for the measured isotope peaks. The data is firstly shown as bar charts for the control and the substrate data. See Figure 5.9 for these graphs.

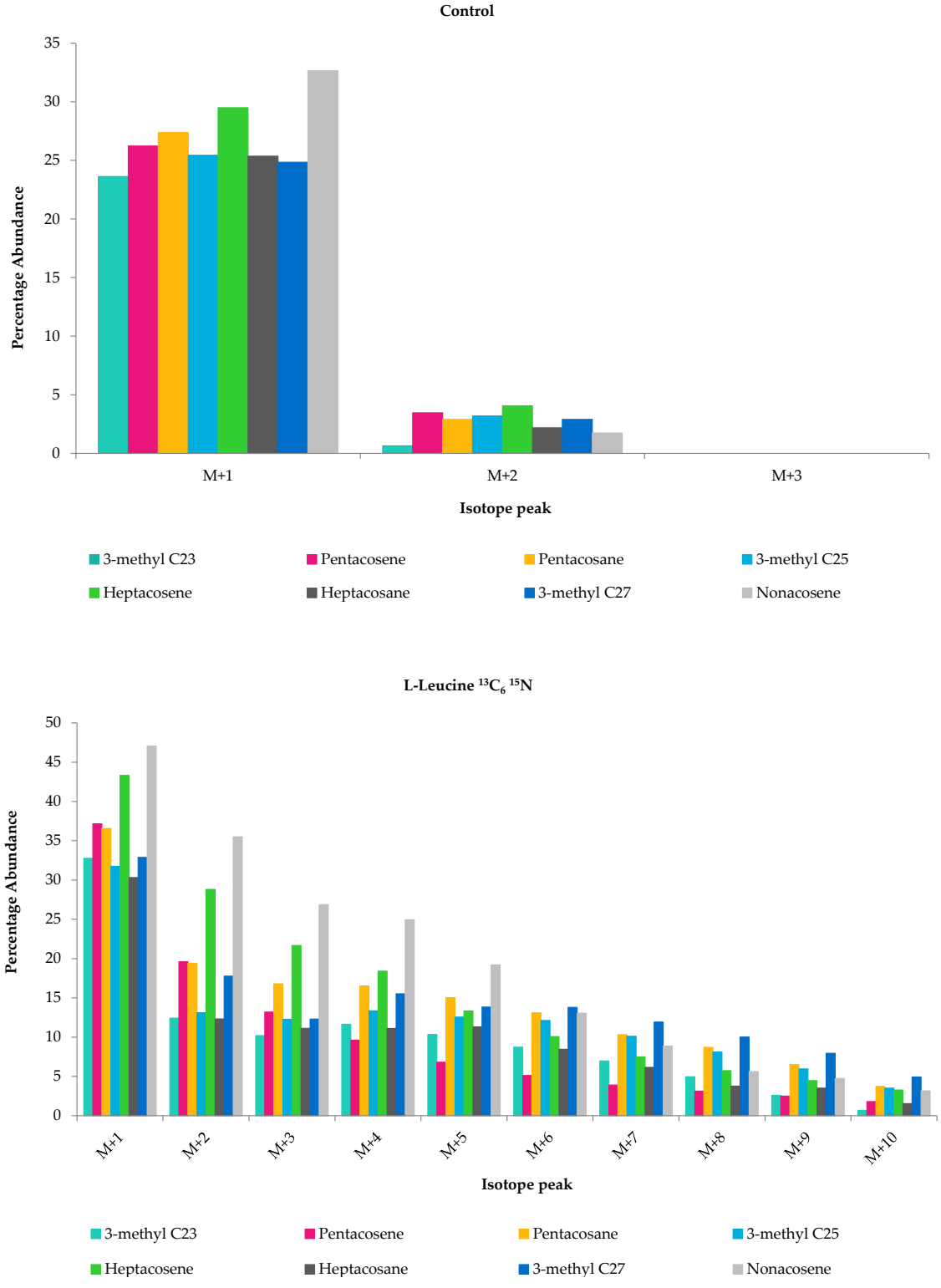


Figure 5.9: Bar chart showing the percentage abundance results of the L-leucine control data and the L-leucine $^{13}C_6$,^{15}N substrate data for the studied isotope peaks for *Formica lemani*.

The difference in the two sets of data can be clearly seen. For the control data there were no $I^{(M+3)}$ peaks present and hence no bars are shown for this isotope peak. For the substrate data there were high percentage values for $I^{(M+10)}$. The substrate data indicates that the percentage abundances for the isotope peaks follow a gradual overall downwards trend for all the compounds, however there are some more subtle differences between the compounds. To explore these trends more thoroughly the substrate data was plotted as a line chart to show the individual compound trends more clearly. For this line chart see Figure 5.10.

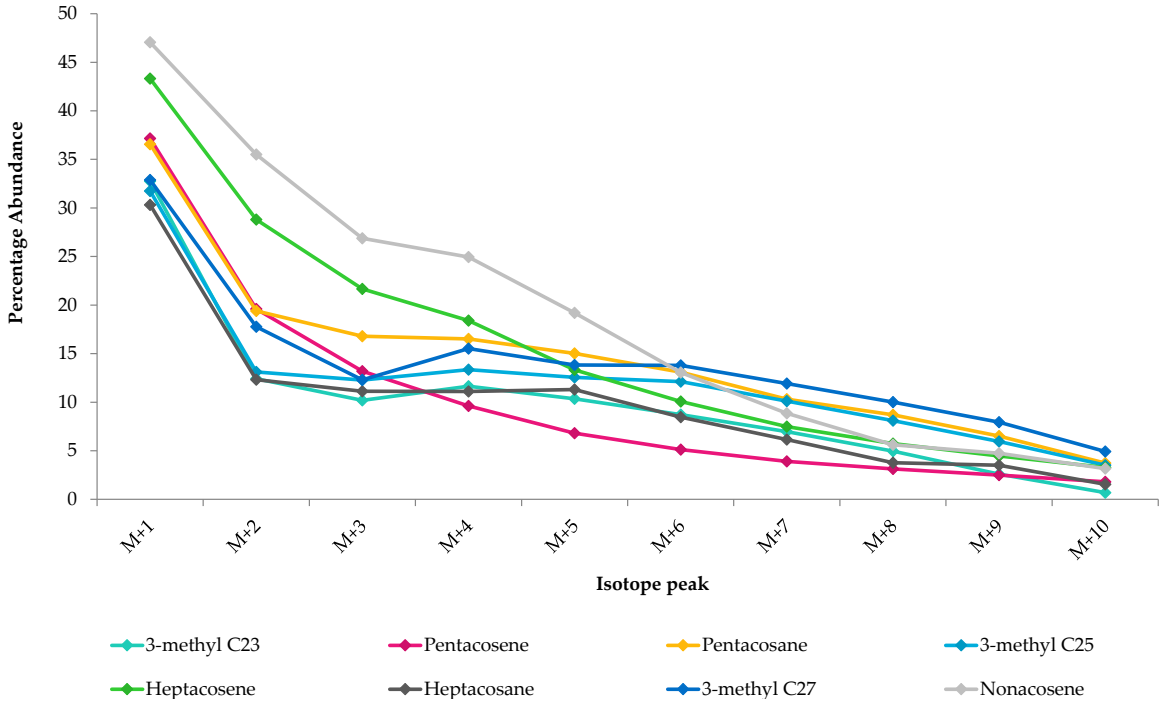


Figure 5.10: Line chart showing the downward trends in percentage abundances for each compound of the L-leucine $^{13}C_6$,^{15}N substrate data for *Formica lemani*.

In the form of a line chart there are more subtle differences that can be seen within the substrate data. Firstly all eight of the studied compounds follow an overall gradual downwards trend. In order to further explore these trends in more detail separate line charts were produced for each type of compound; alkenes, alkanes and methyl-branched hydrocarbons. Error bars representing +/- two standard errors have been added.

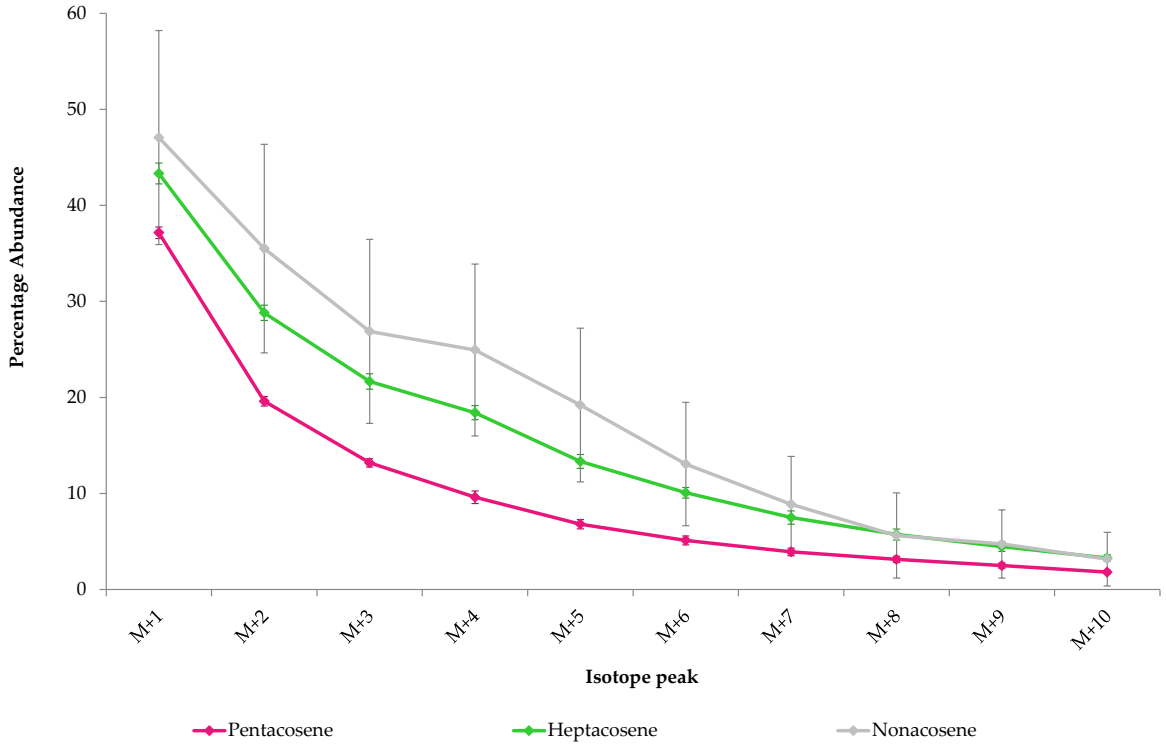


Figure 5.11: Line chart showing the downward trends in percentage abundances for the alkene compounds of the L-leucine $^{13}C_6$,^{15}N substrate data for *Formica lemani*.

Looking at the first of these line graphs; Figure 5.11, it can be seen that the three alkenes all follow the same gradual downward trend; a gentle curve, which, although it starts steeply, becomes much more shallow at a fairly even rate. The error bars are very much the largest for nonacosene. This is likely because this compound has the lowest abundance of the three according to the data sheets, see Appendices C.11-C.14. As previously found the lower the abundance of the compound, the greater the variability, this is generally because the detection of the less abundant compounds may be approaching the limit of detection of the instrument, and therefore it is less acurate. Therefore if an extract is not hydrocarbon rich, the first compounds to become undetectable will be those with the lowest abundance. These will have a value of 0.0%, meanwhile those with a more hydrocarbon rich extract may have a much higher value, resulting in data with a large variation of range and hence a large associated error. In contrast the most abundant compound for this species is pentacosene, and therefore the error for this compound is very low.

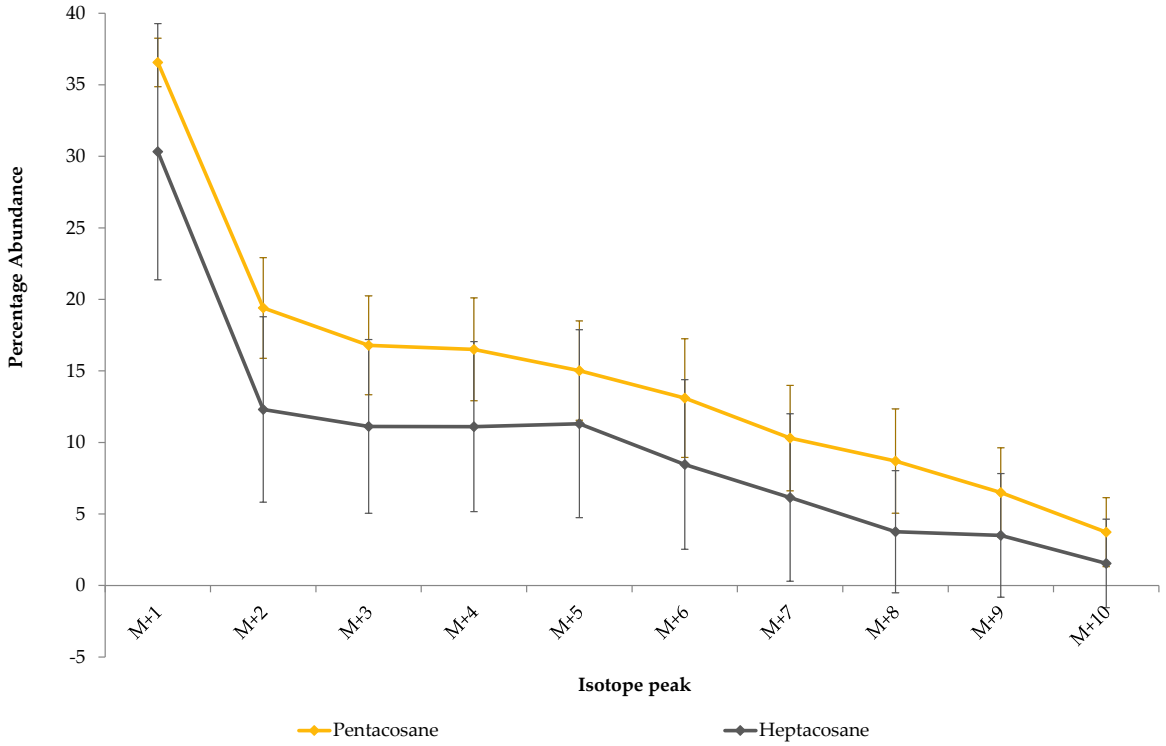


Figure 5.12: Line chart showing the downward trends in percentage abundances for the alkane compounds of the L-leucine $^{13}C_6$,^{15}N substrate data for *Formica lemani*.

The two alkane compounds however follow a slightly different trend. These compounds have a steep decrease from M+1 to M+2, after this the change is a much more gradual decrease. This is in contrast to the alkenes where the decrease was much more of a curve. It should be noted that all straight chain hydrocarbons follow a downward trend with increasing M+n, with the exception of heptacosane (dark grey in Figure 5.12). This compound shows a very slight increase in percentage abundance from $I^{(M+3)}$ and $I^{(M+4)}$ (both 11.1%) to $I^{(M+5)}$ at 11.3%. However the increase is so small (0.2%) that it could be argued that is simply a random fluctuation rather than an indicative trend.

The pattern shown by the methyl-branched hydrocarbons appears to be quite different to that of the straight chain hydrocarbons, see Figure 5.13. Looking at just this data it can be seen that the values for the $I^{(M+1)}$ peaks for these methyl-branched compounds are some of the lowest percentage abundances compared to the other compounds, with value between 31% and 33% compared to 36% and above for other compounds. The

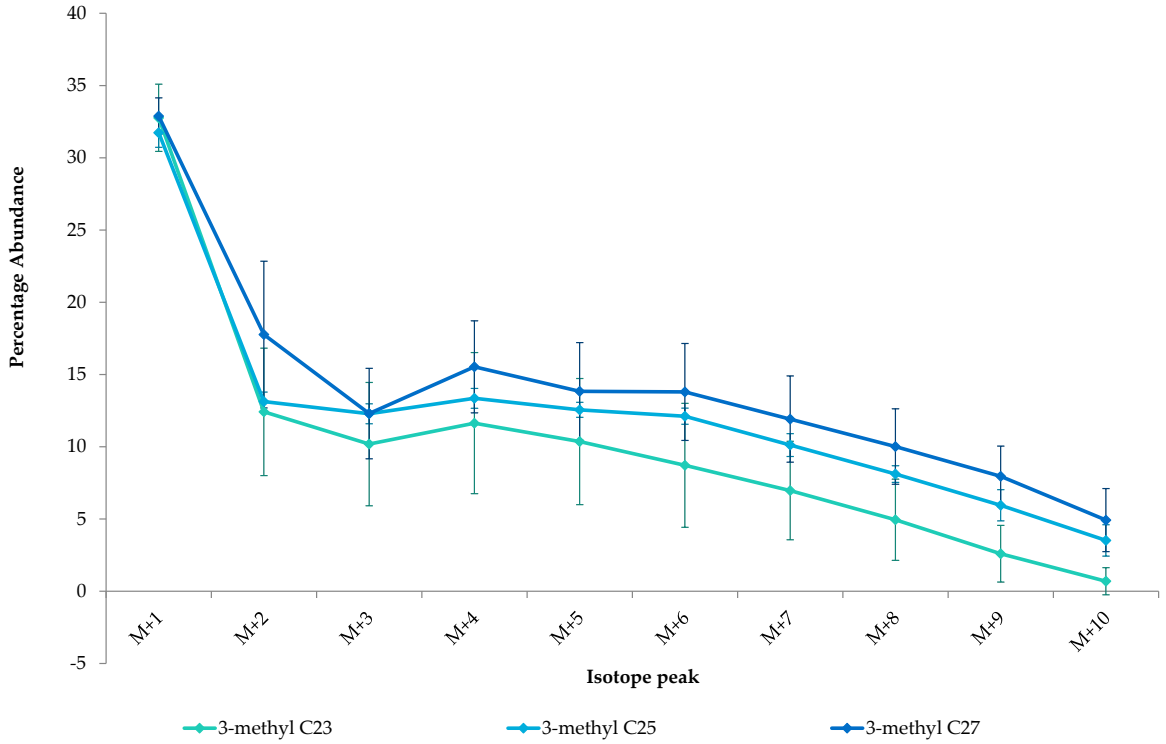


Figure 5.13: Line chart showing the downward trends in percentage abundances for the methyl-branched compounds of the L-leucine $^{13}C_6$,^{15}N substrate data for *Formica lemani*.

percentage abundances for these three compounds fall steeply, following this, each of these compounds shows a slight increase for $I^{(M+4)}$ before again showing a very gradual downwards trend. This increase in percentage abundance for $I^{(M+4)}$ could indicate that there is some significance in a mass increase of 4 Da. For 3-methyl heptacosane, (dark blue), there is in addition to the trend described previously a very slight increase for $I^{(M+6)}$; this may indicate that for these methyl-branched compounds 2 Da are added with each incorporation of labelled amino acid. Hence this results in the even isotope peak masses showing more incorporation than the odd ones.

Whilst the differences observed between compound types are certainly very subtle there is no doubt that differences in incorporation exist between the compound types. This may suggest that there is compound specific incorporation but that these differences are very marginal.

Myrmica sabuleti

Again when looking at the mass spectra for L-leucine $^{13}C_6$,^{15}N and *Myrmica sabuleti* it could be seen that there were large amounts of incorporation. As expected from previous results there was again greater incorporation than that seen for *Formica lemani*. Due to the large amounts of incorporation it was decided to record the intensities of the M+X isotope peaks up to, and including, M+15, as it was at this level that values were seen approaching 0%. Again this was so that any trends within the incorporation for the various compounds could be clearly analysed. However for the purposes of comparing this data against the control, only the peaks for $I^{(M+X)}$, where X=1-3, will be initially presented. See Figure 5.14 for the comparable mass spectra of pentacosene for the control and substrate data.

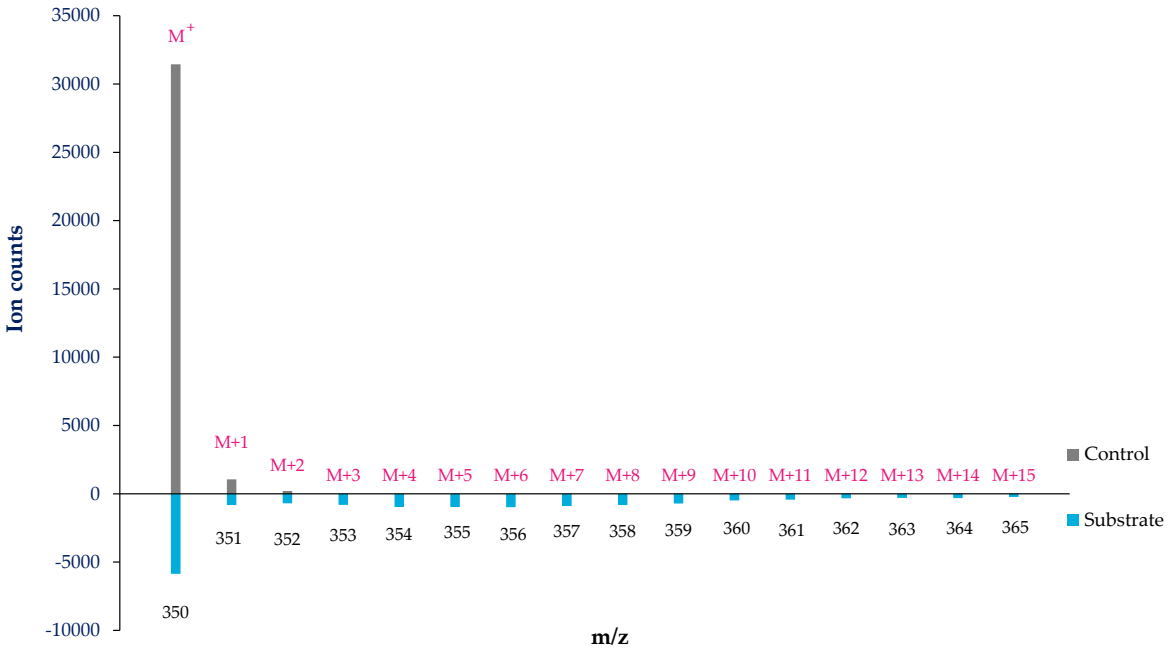


Figure 5.14: Extracted mass spectrum for *Myrmica sabuleti* and the L-leucine $^{13}C_6$,^{15}N substrate experiment showing average comparative ion counts for control and substrate data sets for the compound pentacosene.

It should also be noted that because this was a different colony to that used before; the abundances of the compounds present within the chemical profile was slightly different. This meant that slightly different compounds were selected for analysis compared to

the previous amino acid experiment with this species. Figure 5.15 shows boxplots for the L-leucine $^{13}C_6$,^{15}N substrate for $I^{(M+1)}$, $I^{(M+2)}$ and $I^{(M+3)}$.

The data shows that the variation within the control samples is fairly small, note that for the boxplots representing $I^{(M+3)}$ all the control values are 0.0%. The data for the substrate however is greater, although, with the exception of pentacosane for $I^{(M+1)}$, the range is still fairly small. The boxplots also indicate that for all studied isotope peaks and all studied compounds the percentage abundances of the isotope peaks are much higher for the population which was fed the labelled substrate.

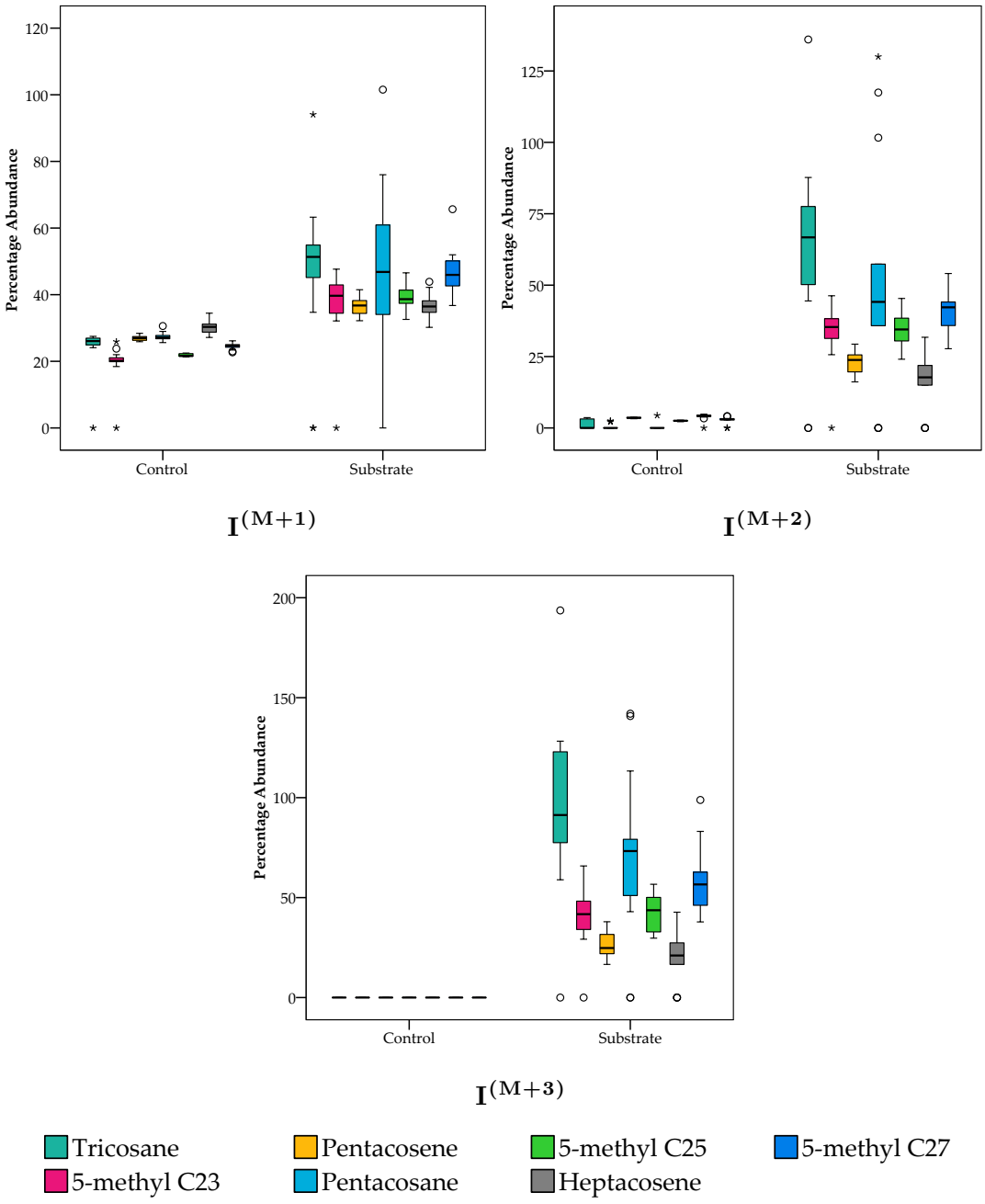


Figure 5.15: Boxplots showing the data for *Myrmica sabuleti* for L-leucine $^{13}C_6$,^{15}N. $I^{(M+1)}$ is on the left, $I^{(M+2)}$ on the right and $I^{(M+3)}$ below.

Figure 5.15 shows an interesting trend in that for all three M+n levels the compounds can be grouped according to type. This observation is the clearest for $I^{(M+3)}$, where the two alkane compounds (shown in turquoise and light blue) are positioned higher up the y axis than the three methyl-branched hydrocarbons (pink, green and dark blue) and the two alkenes (yellow and grey). Figure 5.16 shows the same data as that above, however the compounds have been grouped to make this observation clearer.

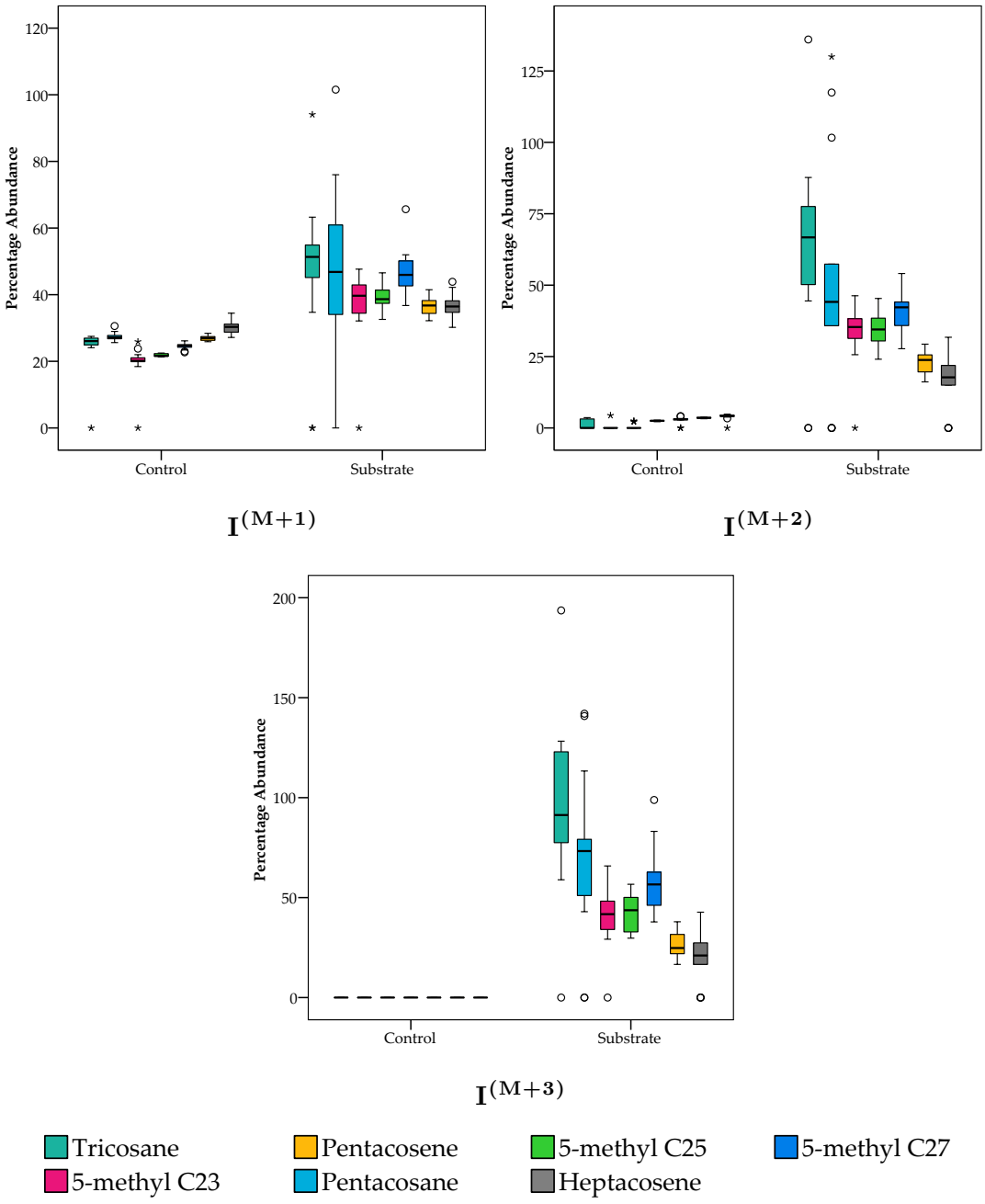


Figure 5.16: Boxplots showing the reordered data for *Myrmica sabuleti* for L-leucine $^{13}C_6$,^{15}N. $I^{(M+1)}$ is on the left, $I^{(M+2)}$ on the right and $I^{(M+3)}$ below.

These boxplots clearly show the differences in incorporation between the compound types; the alkanes generally show the most incorporation, followed by the methyl-branched hydrocarbons, with the alkenes showing the least incorporation. In the same way the data was analysed using a non-parametric statistical test and the following table shows the Mann-Whitney U-test data for *Myrmica sabuleti* and the labelled substrate L-leucine $^{13}C_6$,^{15}N.

Table 5.4: The probability values from the calculated Mann-Whitney U-tests for *Myrmica sabuleti*, for L-leucine $^{13}C_6$,^{15}N, and all three isotope peaks. Note that any p values less than 0.050, and therefore significant, are underlined for ease of reference.

Compound	p value L-leucine $^{13}C_6$,^{15}N		
	$I^{(M+1)}$	$I^{(M+2)}$	$I^{(M+3)}$
Tricosane	<u>0.001</u>	<u>0.000</u>	<u>0.000</u>
5-methyl C23	<u>0.000</u>	<u>0.000</u>	<u>0.000</u>
Pentacosene	<u>0.000</u>	<u>0.000</u>	<u>0.000</u>
Pentacosane	<u>0.001</u>	<u>0.000</u>	<u>0.000</u>
5-methyl C25	<u>0.000</u>	<u>0.000</u>	<u>0.000</u>
Heptacosene	<u>0.000</u>	<u>0.007</u>	<u>0.000</u>
3-methyl C27	<u>0.000</u>	<u>0.000</u>	<u>0.000</u>

As this statistical analysis shows for all isotope peaks and all measured compounds there is a statistical difference between the control and substrate populations. As with *Formica lemani* the data was further analysed to the M+15 level to see if any additional trends could be identified. The graphs shown in Figure 5.17 represent the varying percentage abundances of the ion counts for the measured isotope peaks. The data is firstly shown as bar charts for the control and the substrate data. See Figure 5.17 for these graphs.

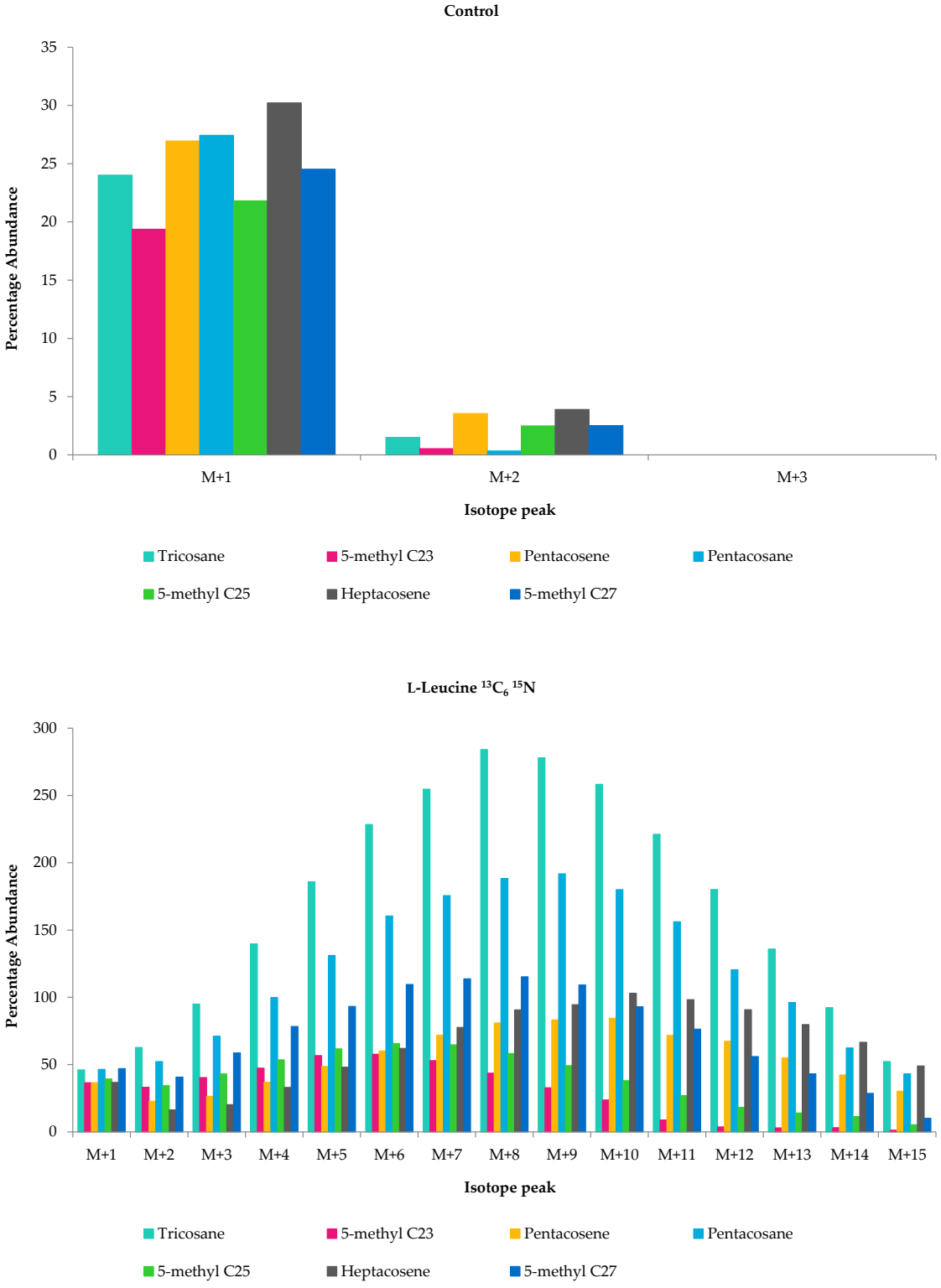


Figure 5.17: Bar chart showing the percentage abundance results of the L-leucine control data and the L-leucine $^{13}C_6$,^{15}N substrate data for the studied isotope peaks and the species *Myrmica sabuleti*.

The data shown in Figure 5.17 shows that once again the control percentage abundance values for $I^{(M+3)}$ are all 0.0%. However the values for the substrate data are totally different. These values show that there are large amounts of incorporation, even for the M+15 isotope peak. Although no further isotope peaks were measured there were measurable amounts of M+18 and above for some compounds and samples. Again to further explore these trends the substrate data was plotted as a line chart to show the individual compound trends more clearly. For this line chart see Figure 5.18.

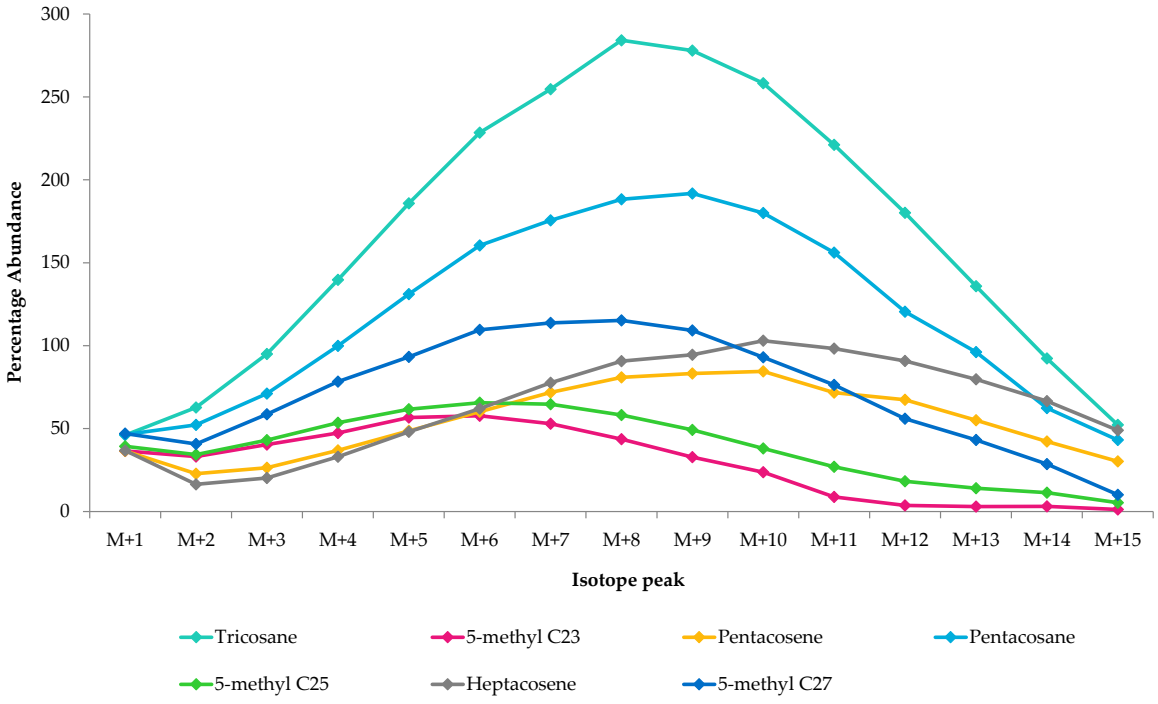


Figure 5.18: Line chart showing the downward trends in percentage abundances for each compound of the L-leucine $^{13}C_6$,^{15}N substrate data for *Myrmica sabuleti*.

As both Figures 5.17 and 5.18 show there is significant incorporation of the labelled substrate into the final hydrocarbons. However there is also a very clear overall trend of incorporation. Whilst a gradual downward trend was seen for *Formica lemani*, for this species the overall trend is very different. As Figure 5.18 shows the trend for this species is a peak with all compounds showing a gradual increase and decrease in percentage abundances. Studying these trends in even greater detail reveals that the

shape of the curve is shifted slightly for the various compounds. In order to explore this in more detail the line graphs were plotted separately according to compound types. Error bars representing +/- two standard errors were also added.

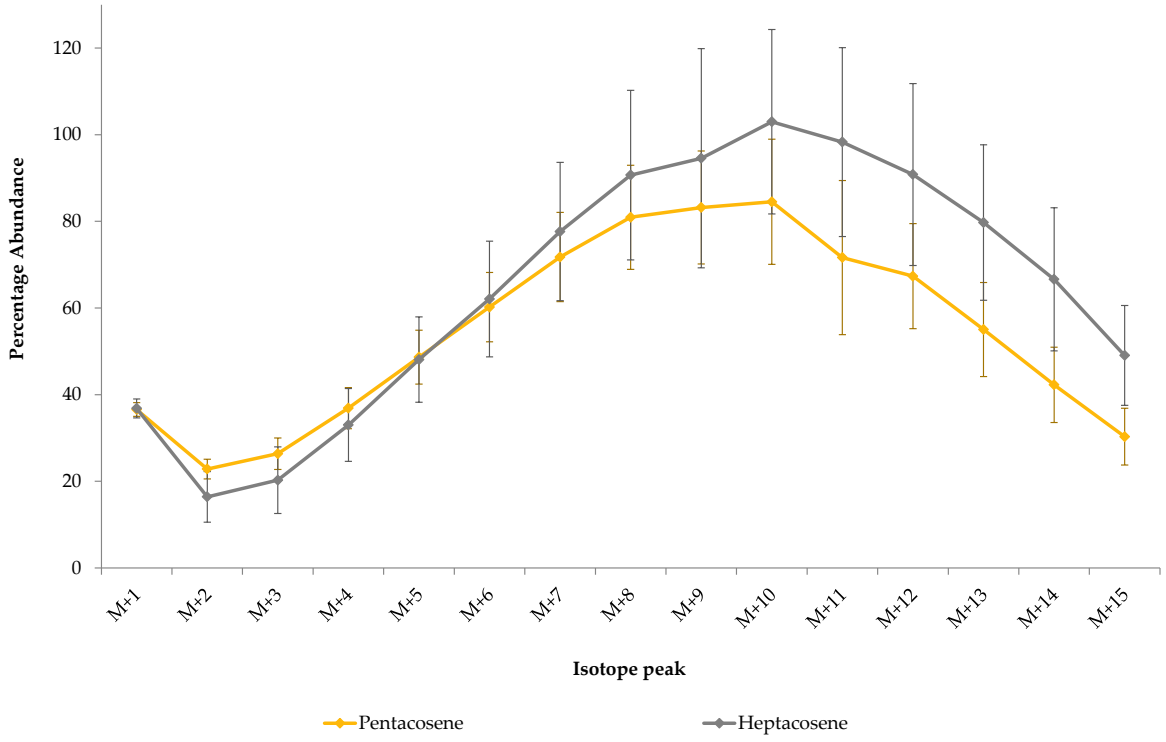


Figure 5.19: Line chart showing the downward trends in percentage abundances for the alkenes of the L-leucine $^{13}C_6$,^{15}N substrate data for *Myrmica sabuleti*.

Firstly the alkene compounds are shown in Figure 5.19. These compounds (pentacosene, shown in yellow and nonacosene shown in grey) both show a very similar overall trend. Initially they dip from $I^{(M+1)}$ to $I^{(M+2)}$ and after this they both follow a fairly steep upward trend until they both reach a maximum percentage abundance corresponding to the $I^{(M+10)}$ peak. Following these maxima they both gradually decrease again at a very similar rate. The error bars associated with these compounds are relatively large and hence there is some overlap between them.

In contrast the two alkane compounds (tricosane in turquoise and pentacosane in green) shown in Figure 5.20 indicate a peak shape which is fairly centrally positioned. The

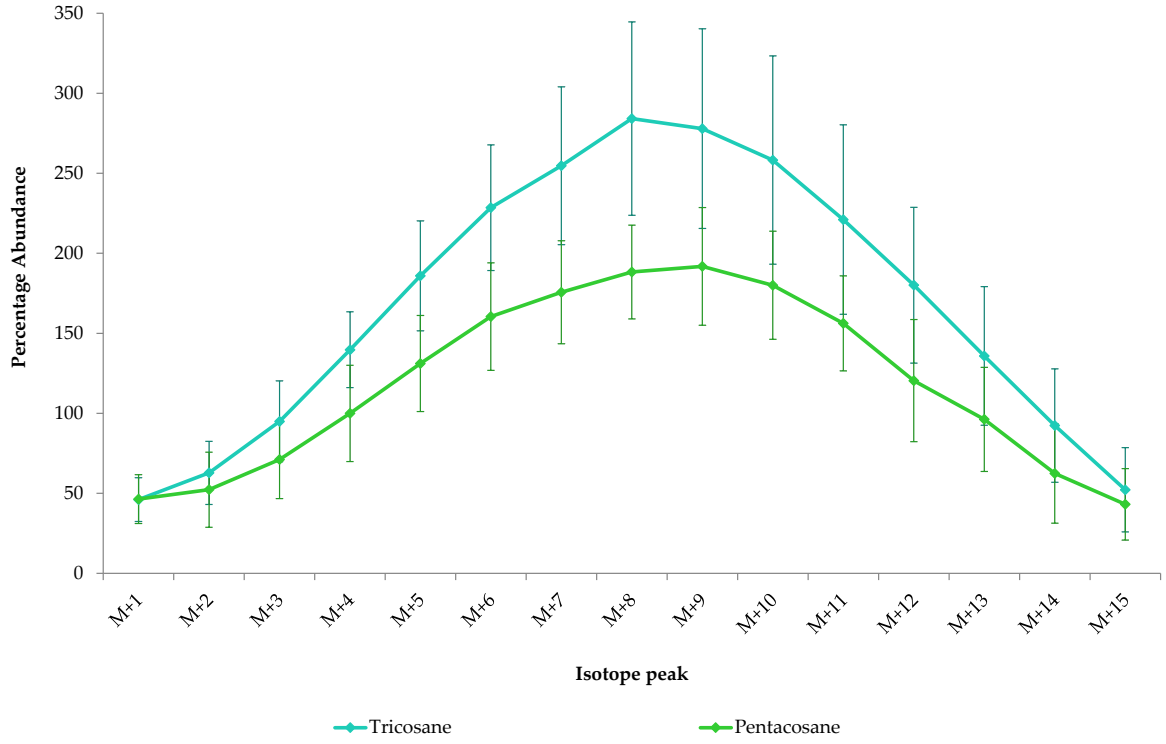


Figure 5.20: Line chart showing the downward trends in percentage abundances for the alkanes of the L-leucine $^{13}C_6$,^{15}N substrate data for *Myrmica sabuleti*.

percentage abundance values gradually increase with maxima corresponding to $I^{(M+8)}$ for tricosane and $I^{(M+9)}$ for pentacosane. The values for $I^{(M+15)}$ are very similar to those for $I^{(M+1)}$ which gives both of these compounds a fairly symmetrical trend, with the rate of increase similar to the rate of decrease.

Finally the methyl-branched compounds as shown in Figure 5.21. Firstly it is evident that 5-methyl C23 and 5-methyl C25 (pink and light blue) both follow a very similar pattern which can be described as having a shape similar to that observed for the alkenes however it is slightly shifted to the left. Again both of these compounds show a slight initial dip before both having a maximum percentage incorporation value at $I^{(M+6)}$. Following this both compounds show a gradual downwards trend at a similar rate before the rate of decrease becomes much less at around $I^{(M+12)}$, before the values begin to approach 0.0% for $I^{(M+15)}$. Showing a slightly different shape is the final compound 5-methyl C27 (shown in dark blue). This shows an initial dip similar

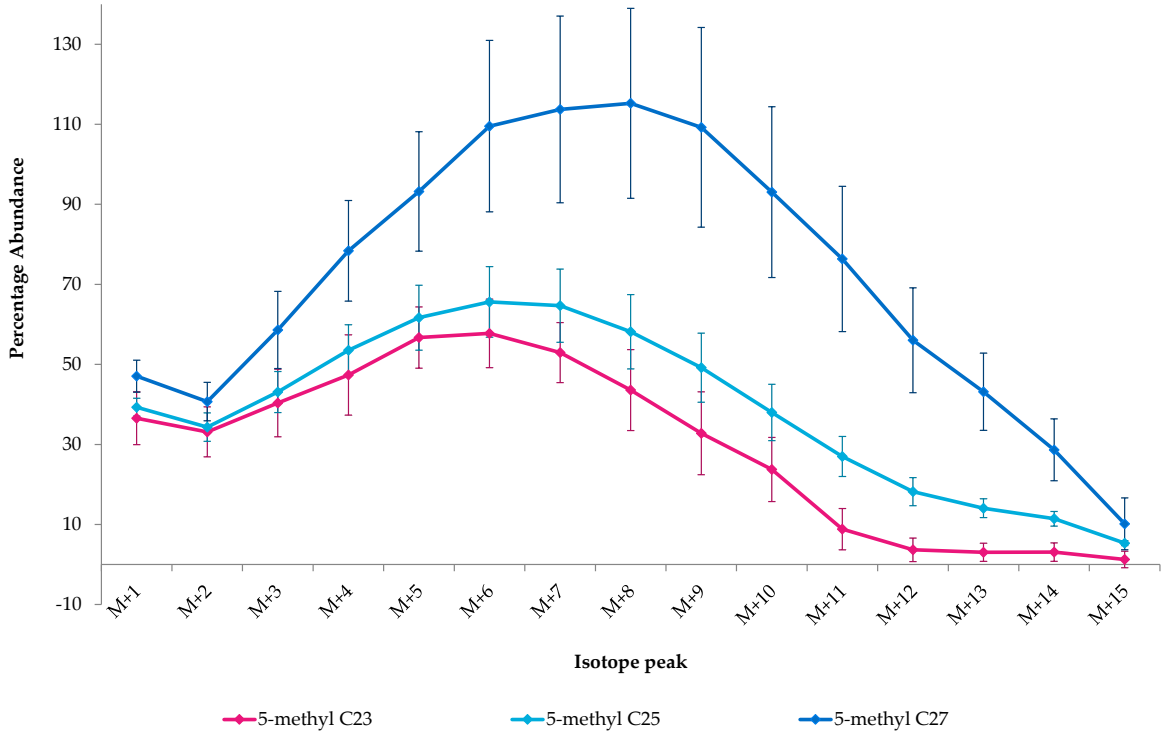


Figure 5.21: Line chart showing the downward trends in percentage abundances for the methyl-branched compounds of the L-leucine $^{13}C_6$,^{15}N substrate data for *Myrmica sabuleti*.

to that shown by the other methyl-branched compounds, but has a maximum value at $I^{(M+8)}$ with a greater rate of increase and decrease. There is also no change in the rate of decrease as seen for the other methyl-branched compounds. However it should be noted that unlike the alkanes, the shape of the curve is unsymmetrical, with values nearing 0.0% for $I^{(M+15)}$; a trend not observed for the alkanes, where even at $I^{(M+15)}$ incorporation was still close to 50%.

5.3.1.3 Discussion

L-leucine 5,5,5-d_3

Analysis of the literature shows that it is already known that valine and leucine are key precursors in the formation of 2-methyl alkanes [2]. However neither of the studied species produce compounds of this type. Firstly looking at the results from the experiments involving L-leucine 5,5,5-d_3 it can be seen that there was a small amount of incorporation observed. Statistical significance was seen for pentacosene at the $I^{(M+1)}$ level for *Formica lemani* and within the internally branched methyl compounds for *Myrmica sabuleti* at the $I^{(M+1)}$ and $I^{(M+3)}$ levels. However based on these results it cannot be said that incorporation was widespread. Analysis of the literature indicates that incorporation should only be expected for 2-methyl alkanes [2]. So, although not conclusive, any incorporation does not fit with what is understood from the literature. Therefore this could mean that these species are able to breakdown the labelled leucine into another biologically important molecule. This product is then used in subsequent biosynthesis to yield hydrocarbons with labels present.

In order to determine whether this is the case it is necessary to study the metabolic breakdown of leucine. In the first step of the process leucine is transaminated to form the α-ketoacid; α-ketoisocaproate. This compound is then subsequently oxidatively carboxylated to the corresponding CoA derivative; isovaleryl-CoA. This compound is then oxidised via flavin adenine dinucleotide (FAD). At this point the terminal methyl group is first carboxylated, following this subsequent hydration occurs to form a tertiary alcohol. This is then thiolysed to form acetoacetate and acetyl-CoA [5, 6]. See Figure 5.22 which shows this reaction scheme.

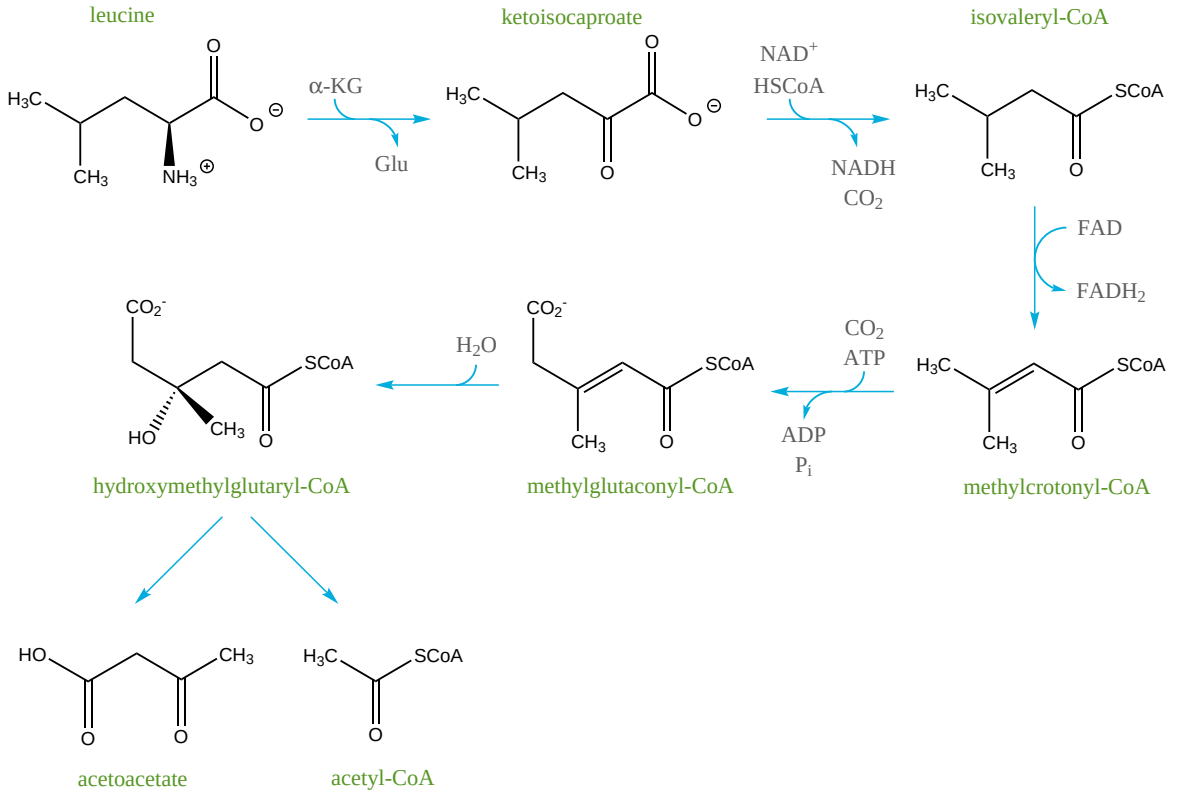


Figure 5.22: Reaction scheme showing the breakdown of leucine to form acetoacetate and acetyl-CoA, adapted from [5, 6].

As this degradation pathway shows, one of the metabolic products of the breakdown of leucine is acetyl-CoA. This compound is known to lengthen fatty acid chains and as such is used during the biosynthesis of a variety of compounds, including alkenes, alkanes and methyl-branched alkanes. However although acetyl-CoA is produced in this reaction it does not necessarily contain any labelled deuterium atoms, as these may be lost during the process. It therefore may not be the source of the labels that are seen in some of the resulting biosynthesised compounds.

In order to determine the source of the extra isotopes detected in the biosynthesised hydrocarbons, this degradation pathway must be considered with the substrate labels indicated. Figure 5.23 shows this degradation pathway with the position of the deuterium atoms indicated. As can be seen from this scheme there is an acetyl-CoA molecule produced from each metabolised molecule of L-leucine 5,5,5-d_3, however this does not contain any deuterium atoms. Therefore this cannot be the source of the ex-

tra isotopic mass detected in the biosynthesised hydrocarbons, instead this must arise from a further metabolic pathway.

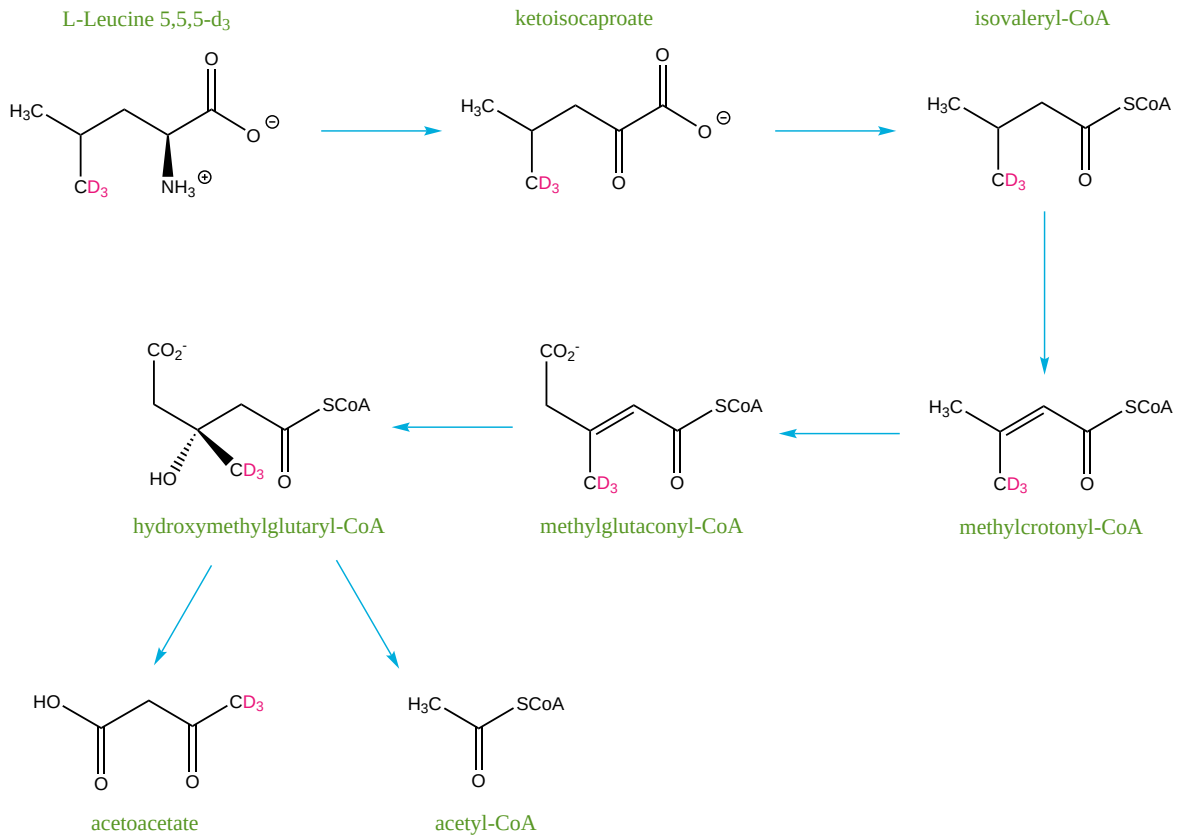


Figure 5.23: Reaction scheme showing the breakdown of L-leucine 5,5,5-d_3 with the position of the deuterium labels shown, adapted from [5, 6].

The other reaction product shown by the degradation pathway shown in Figure 5.23 is a labelled acetoacetate, this is a ketone body, and can be used as a source of energy [7]. However in order to utilise ketone bodies as energy sources an additional pathway exists in order to break them down further. Figure 5.24 shows this pathway, the positions of the labels obtained from the metabolic breakdown in 5.24 are included.

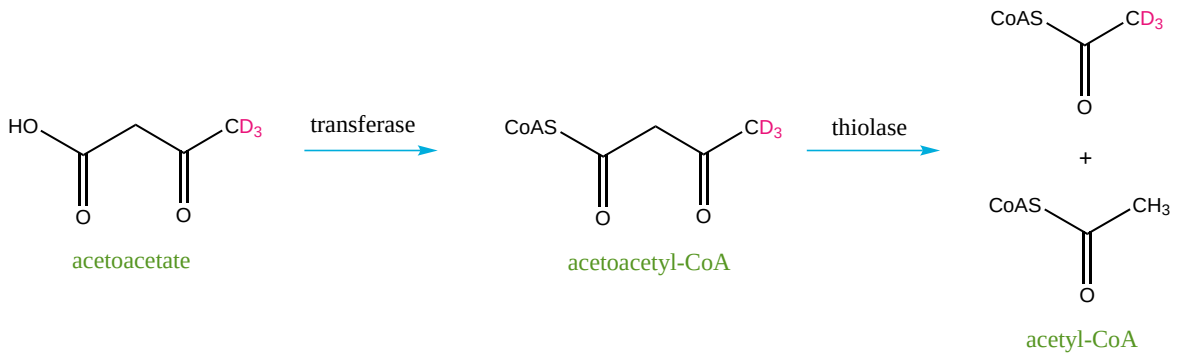


Figure 5.24: Reaction scheme for the breakdown of acetoacetate into acetyl-CoA with the position of the deuterium labels shown, adapted from [7, 8].

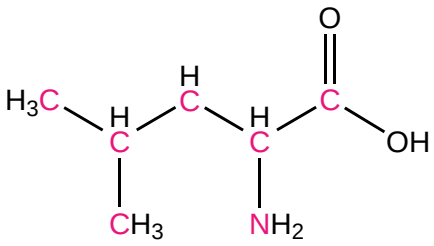
As can be seen from this reaction scheme the acetoacetate is first converted to acetoacetyl-CoA via the CoA transferase enzyme, from this the acetoacetyl-CoA is further broken down using a thiolase enzyme in order to produce two acetyl-CoA units. As indicated by the schematic shown in Figure 5.24 one of the resulting acetyl-CoA molecules produced contains the deuterium labels from the original leucine molecule. As Sections 1.2.2.2 and 1.2.2.3 described acetyl-CoA can be used for a variety of purposes. It can be used in the *de novo* synthesis of fatty acids, or can be used to lengthen a fatty acid prior to it being converted into an alkene or alkane. If it is assumed that an acetyl-CoA molecule is used which contains a CD_3 in the position indicated in Figure 5.24, then as the mechanisms contained within Sections 1.2.2.2 and 1.2.2.3 indicate, either usage would result in one or more deuterium atoms present being incorporated into the final hydrocarbon.

As this incorporation is as a result of various metabolic pathways, it may explain why there is no clear overall trend to the results. For *Formica lemani*, labelling was evident when studying the value of $I^{(M+2)}$ for several compounds, for *Myrmica sabuleti* it was only seen for pentacosene at the $I^{(M+1)}$ level. It is impossible to say why there appears to be a difference between the two species. Unfortunately it is not possible to determine the position of the resulting deuterium label within the biosynthesised hydrocarbons as deuterium is not detected via proton NMR techniques. However these results support previous research which suggests that ants are not able to metabolise leucine into

propionate, as very clear, conclusive incorporation is not observed for methyl-branched hydrocarbons. Instead any deuterium which is incorporated into the biosynthesised hydrocarbons results from minor metabolic pathways; most likely from the formation and subsequent use of labelled acetyl-CoA.

L-leucine $^{13}C_6$,^{15}N

In contrast to the results observed for L-leucine 5,5,5-d_3, the results for L-leucine $^{13}C_6$,^{15}N were extremely clear. For both species there was widespread incorporation into all compounds with the exception of heptacosane for *Formica lemani* at the $I^{(M+2)}$ level. The structure of this labelled substrate is shown below, see Figure 5.25.



L-Leucine $^{13}C_6$,^{15}N

Figure 5.25: The structure of L-leucine $^{13}C_6$,^{15}N, the position of the labelled atoms are shown in pink.

As can be seen from the structure of this labelled substrate there are six labelled atoms, excluding the nitrogen atom. Each contributes 1 Da, therefore full incorporation would add 6 Da to the labelled hydrocarbon. However as previously described this labelled substrate is only used as a direct precursor for the biosynthesis of 2-methyl alkanes [2]. Therefore the observed incorporation must once again be due to the breakdown of this compound into more biologically useful molecules as seen in Figure 5.26.

As the schematic of the resulting degradation pathway shows, one molecule of labelled acetoacetate and one molecule of labelled acetyl-CoA is produced. As can be seen from Figure 5.26, the labelled acetoacetate is 3 Da heavier whilst the acetyl-CoA contains

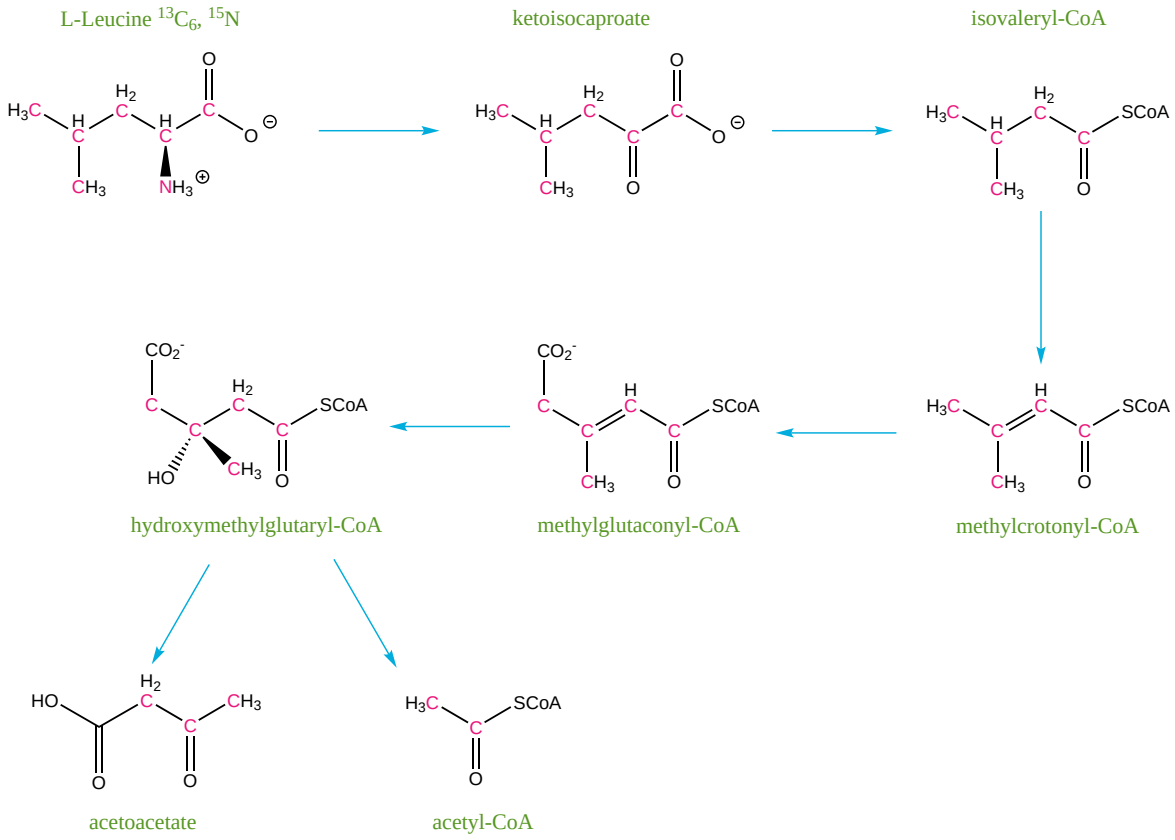


Figure 5.26: Reaction scheme showing the breakdown of L-leucine $^{13}C_6$,^{15}N with the position of the isotopic labels shown in pink, adapted from [5, 6].

two Daltons of extra mass. As observed from the data for the L-leucine $^{13}C_6$,^{15}N experiment there was a trend that seemed to favour the incorporation of even numbers of mass units. For *Formica lemani* the line chart showing the incorporation trends (Figure 5.10) indicated that despite the overall downward trend observed, there were several compounds that showed a less steep downward trend, or even an increase, with even numbered $I^{(M+X)}$ levels, compared to odd. This can be seen clearly in Figure 5.13 which shows the methyl-branched hydrocarbons. In other words there was a general bias towards greater incorporation for $I^{(M+X)}$ where in particular X=2, 4, 6 for both species. This observation, coupled with the fact that each breakdown of a labelled L-leucine $^{13}C_6$,^{15}N molecule yields a doubly labelled acetyl-CoA, which is subsequently 2 Da heavier, may indicate that it is the possible incorporation of this molecule which is responsible for this trend. Whilst the results suggest that this may be a likely cause it is

worth considering other routes. As previously discussed further metabolic breakdown of acetoacetate into acetyl-CoA is known to occur [7, 8] and this further metabolism should be considered with the labelled acetoacetate product from Figure 5.26. Figure 5.27 shows this subsequent metabolic pathway, with the positions of the isotopic labels clearly shown on the resulting acetyl-CoA molecules. See 5.27.

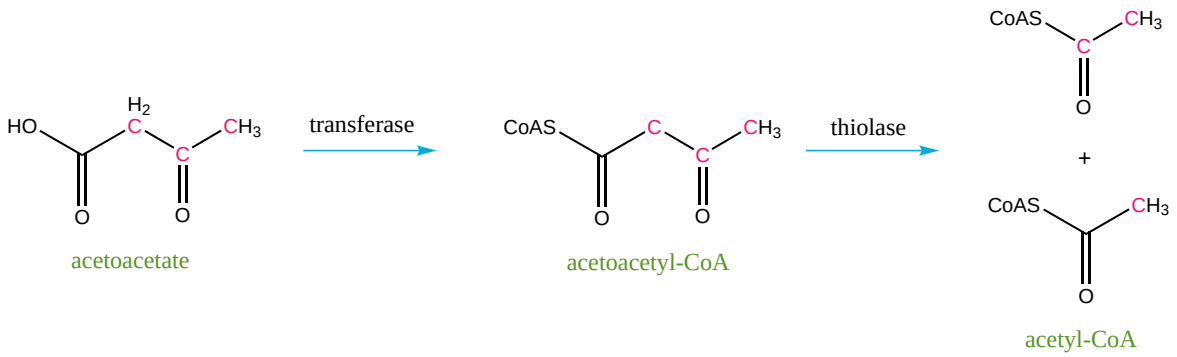


Figure 5.27: Reaction scheme for the breakdown of acetoacetate into acetyl-CoA with the position of the ^{13}C labels shown, adapted from [7, 8].

Therefore the subsequent breakdown of acetoacetate yields a singularly labelled acetyl-CoA and a doubly labelled acetyl-CoA. The metabolism of the labelled L-leucine $^{13}C_6$,^{15}N molecule also yields a doubly labelled acetyl-CoA molecule. As Sections 1.2.2.2, 1.2.2.3, and 1.2.2.4 described, acetyl-CoA can be used during the biosynthesis of alkenes, alkanes or methyl-branched alkanes. See the above mentioned sections for reference. As the mechanisms contained within these sections indicate, each incorporation of a singularly labelled acetyl-CoA would increase the final mass of the resulting hydrocarbon by 1 Da. In the same fashion each incorporation of a doubly labelled acetyl-CoA would increase the final mass of the resulting hydrocarbon by 2 Da. However as many acetyl-CoA units can be used to lengthen the hydrocarbon chain the resulting mass increase of the hydrocarbon could be far greater. As shown each metabolism of labelled leucine yields a doubly labelled acetyl-CoA and a triply labelled acetoacetate, which can be further broken down to form a doubly labelled and a singularly labelled acetyl-CoA. Given that there are more doubly labelled breakdown products it is not surprising that there is a bias towards even amounts of extra mass.

With both species demonstrating a considerable mass increase for both odd and even amounts of mass, it is likely that both of these metabolic routes are used. However what this analysis does not explain is why for *Myrmica sabuleti* the incorporation trend was a peak and for *Formica lemani* it was a gradual downwards trend. The simplest explanation for the differences seen is likely down to the amount of food consumed. The raw data for *Myrmica sabuleti* shows that the ion counts were much greater than that for *Formica lemani* suggesting that there was overall greater incorporation for this species. Again this could be due to the utilisation of the substrate, related to natural supplies of biologically important molecules or the health of the colony. On the other hand it could simply be that *Myrmica sabuleti* consume more food or have a faster rate of hydrocarbon turnover. Once again more research would be required to determine what the likely cause of this is, however this is a trend that has been seen in previous experiments; that the quality of the data is not as good for *Formica lemani* as it is for *Myrmica sabuleti*.

As previously mentioned there were subtle differences for the various compound types within each species. For *Formica lemani* and the substrate L-leucine $^{13}C_6$,^{15}N, the three methyl-branched compounds showed a similar trend with slight increases in the overall downward trend. See Figure 5.13 for this data. By applying the insight gained from the exploration of the mechanisms above it is possible to understand further the trends which are observed. As previously discussed the biosynthesis of a methyl-branched hydrocarbon is down to the formation of the branch initially, via methylmalonate, followed by further elongating the chain using acetate units. See Section 1.2.2.4 for further reference. This therefore means that the longer the chain the more elongation, via acetate units, that is required. It is therefore unsurprising that the longest chain length shows the most undulating downward trend with a very definite increase from $I^{(M+2)}$ (12.3%) to $I^{(M+4)}$ (15.5%). This is followed by $I^{(M+5)}$ and $I^{(M+6)}$ showing the same average percentage abundance when this value is expected to be falling. In the same way the shortest chain shows this trend in a much more subtle fashion with

only one increase in mass from $I^{(M+2)}$ (10.2%) to $I^{(M+4)}$ (11.6%). Following this the downward trend continues at a consistent gradient. The differences in these trends for the various chain lengths, as alluded to, is therefore likely because the longer chain lengths are more likely to incorporate more doubly labelled units. They are therefore more likely to show this bias towards mass increases of an even number. It is however unknown why $I^{(M+4)}$ appears to be so significant.

In the same way this pattern is repeated for the alkenes, that is the longer the chain length the more tendency there is for an obvious change in the rate of the downward trend at the $I^{(M+4)}$ level compared to $I^{(M+3)}$. See Figure 5.11. In other words pentacosene shows a very smooth curve downwards curve, whilst nonacosene shows a value for $I^{(M+4)}$ that is almost the same as that seen for $I^{(M+3)}$, (24.9% compared to 26.9% respectively). The reasoning for this is likely as before; the longer the chain length the greater the likelihood of having multiple doubly labelled acetate units incorporated.

Finally the trend for the alkanes is not as clear. For these compounds there does not seem to be the same bias towards even numbers of mass incorporation; for heptacosane there is an almost level trend seen for $I^{(M+2)}$ to $I^{(M+5)}$, whereby very little change is seen.

The trend for *Myrmica sabuleti* was totally different with the incorporation showing an overall 'peak' shape incorporation, that is the levels generally rose, peaked, and then decreased. However as suggested previously there were subtle differences seen for the various compound types. Looking at these trends once again with the added insight of the potential mechanisms may help to reveal possible causes for these differences. Figure 5.18 shows that the three compounds types showed three slightly different peak shaped patterns. The alkanes were symmetrical peaks with an even rate of increase and decrease. The alkenes were skewed to the right (maxima with pronounced initial dips followed by a less gradual increase than decrease). The methyl-branched hydrocarbons

were skewed to the left with a fairly even rate of increase and decrease.

For the alkanes, it would be expected that pentacosane would show more incorporation as it is the longer chain length, however this is not the case. Instead it should be noted that the ion counts for these compounds were low compared to the ion counts for the other compounds; *Myrmica sabuleti* possessing a chemical profile which is not particularly rich in alkanes. See Appendices C.17-C.21. The lower ion counts lead to greater inaccuracy in the measurement of the data and hence this could be why the obvious trend is not seen. For the other classes of compounds; alkenes and methyl-branched hydrocarbons this obvious expectation is observed and the longer chain lengths show the greater incorporation.

It is possible that the patterns observed are down to the biosynthetic routes utilised, however this is mostly conjecture unless further research is performed in this area. It is possible that the skew of the alkenes to the right or to higher levels of $I^{(M+X)}$ is because the biosynthesis of these compounds is utilising a shorter starting material compared to that of the alkanes. This therefore means, that in theory, the shorter starting material would require more subsequent elongation than a potentially longer starting material used for the alkanes. This in turn may indicate the use of a different starting material which may mean a saturated fatty acid for the alkanes and an unsaturated fatty acid for the alkenes. However this can only be theorised as an explanation.

The assymterical distribution of the peak for the methyl-branched alkanes could, in the same way, be due to the fact that some of the elongation occurs via a propionate molecule, in the form of methyl malonate. Therefore in theory, less elongation can occur via acetate molecules. However if this is the case it would be expected that the formation of these compounds would be via *de novo* formation, i.e. the chain length built up from a very short chained molecule, therefore much higher levels of incorporation would be expected and this is not the case. In fact the opposite is true,

the methyl-branched compounds show overall the least incorporation of all compound types.

As the complexity of this work shows there are many further areas that need to be explored and this work, whilst providing an insight, is only really a starting point. What this data does show however is that the incorporation of the labelled leucine is not by any means uniform with different species and different compounds showing varying levels of incorporation and different trends.

5.3.2 Valine

5.3.2.1 DL-valine d_8

The following data shows the results for the feeding experiments using the labelled substrate DL-valine d_8.

Formica lemani

For this experiment the number of control samples for this species was 28, and the number of substrate samples was 22. These boxplots, shown in Figure 5.28, show that there does not appear to be much difference between the two groups for either isotope. The biggest variation in data is seen for the $I^{(M+1)}$ data, whilst that for $I^{(M+3)}$ shows very little variation, this is because most of the values for this isotope peak are 0.0%, with other values being represented by outlier markers.

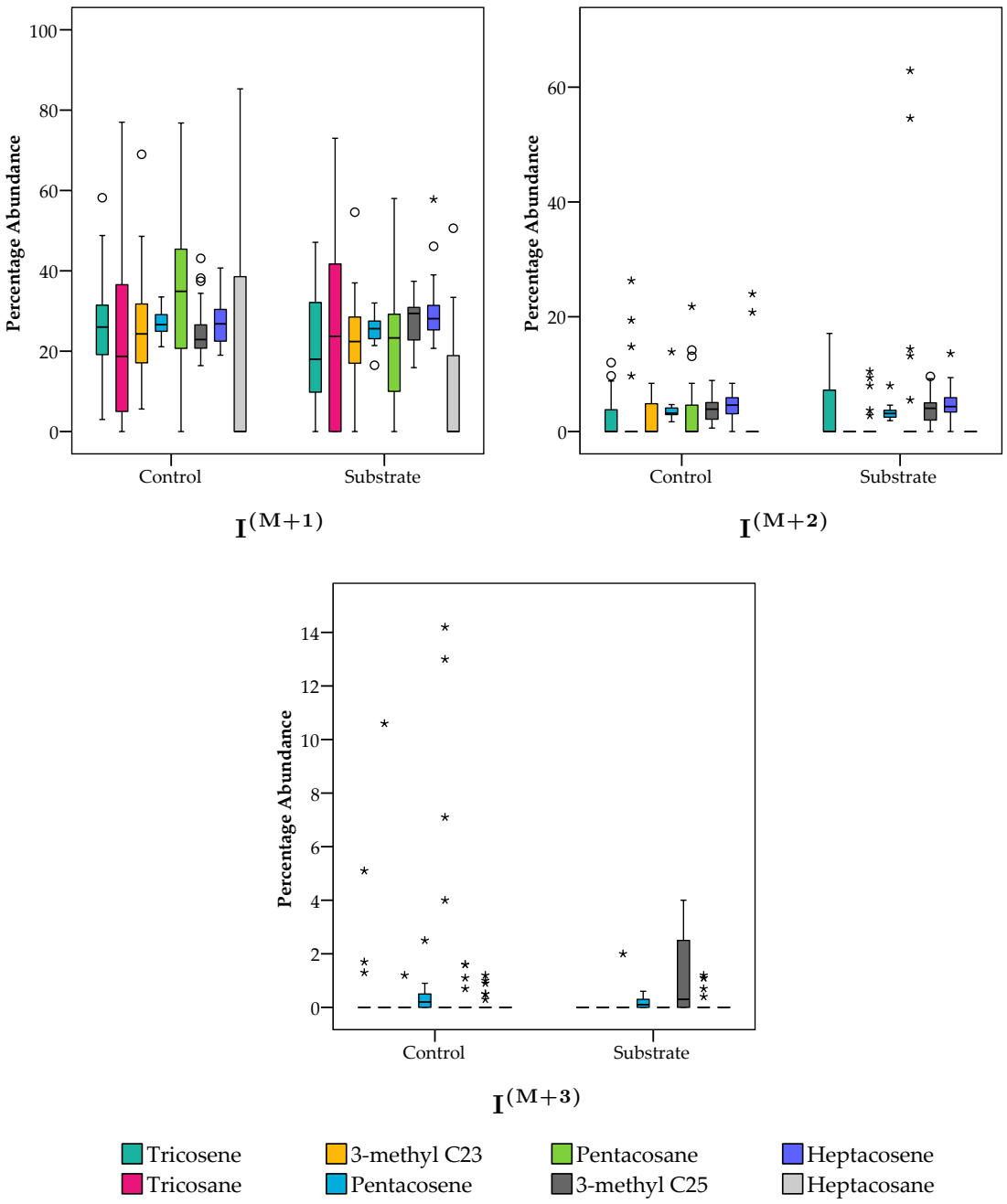


Figure 5.28: Boxplots showing the data for *Formica lemani* for DL-valine d_8. $I^{(M+1)}$ is on the left, $I^{(M+2)}$ on the right and $I^{(M+3)}$ below.

Table 5.5 shows that there is very little significance between the two groups, with the only differences being seen for the compound 3-methyl C25 at the $I^{(M+1)}$ and $I^{(M+3)}$ level and the compound 3-methyl C23 at the $I^{(M+3)}$ level. Although these results are not very clear they do indicate that the DL-valine d_8 substrate may possibly only be used to biosynthesise methyl-branched compounds, and when this substrate is utilised,

Table 5.5: The probability values from the calculated Mann-Whitney U-tests for *Formica lemani*, for DL-valine d_8, and all three isotope peaks. Note that any p values less than 0.050, and therefore significant, are underlined for ease of reference. Any p values less than 0.050 which are italicised may be disregarded and are due to the median rank of the control being greater than that of the substrate group.

Compound	p value DL-valine d_8		
	$I^{(M+1)}$	$I^{(M+2)}$	$I^{(M+3)}$
Tricosene	0.080	0.326	0.167
Tricosane	0.290	*0.046*	0.560
3-methyl C23	0.339	0.125	<u>0.044</u>
Pentacosene	0.106	0.159	0.198
Pentacosane	*0.005*	0.358	0.089
3-methyl C25	<u>0.031</u>	0.490	<u>0.004</u>
Heptacosene	0.109	0.355	0.384
Heptacosane	0.195	0.298	1.000

3 Da of mass are incorporated into the final hydrocarbon.

Myrmica sabuleti

For this experiment the number of control samples for this species was 27, and the number of substrate samples was 24. As Figure 5.29 indicates there does not appear to be any difference between the groups for $I^{(M+1)}$, and the data is fairly consistent with not much variation. The data for $I^{(M+2)}$ and $I^{(M+3)}$ shows that the larger variation is associated with the results for the substrate, with the control showing very little variation.

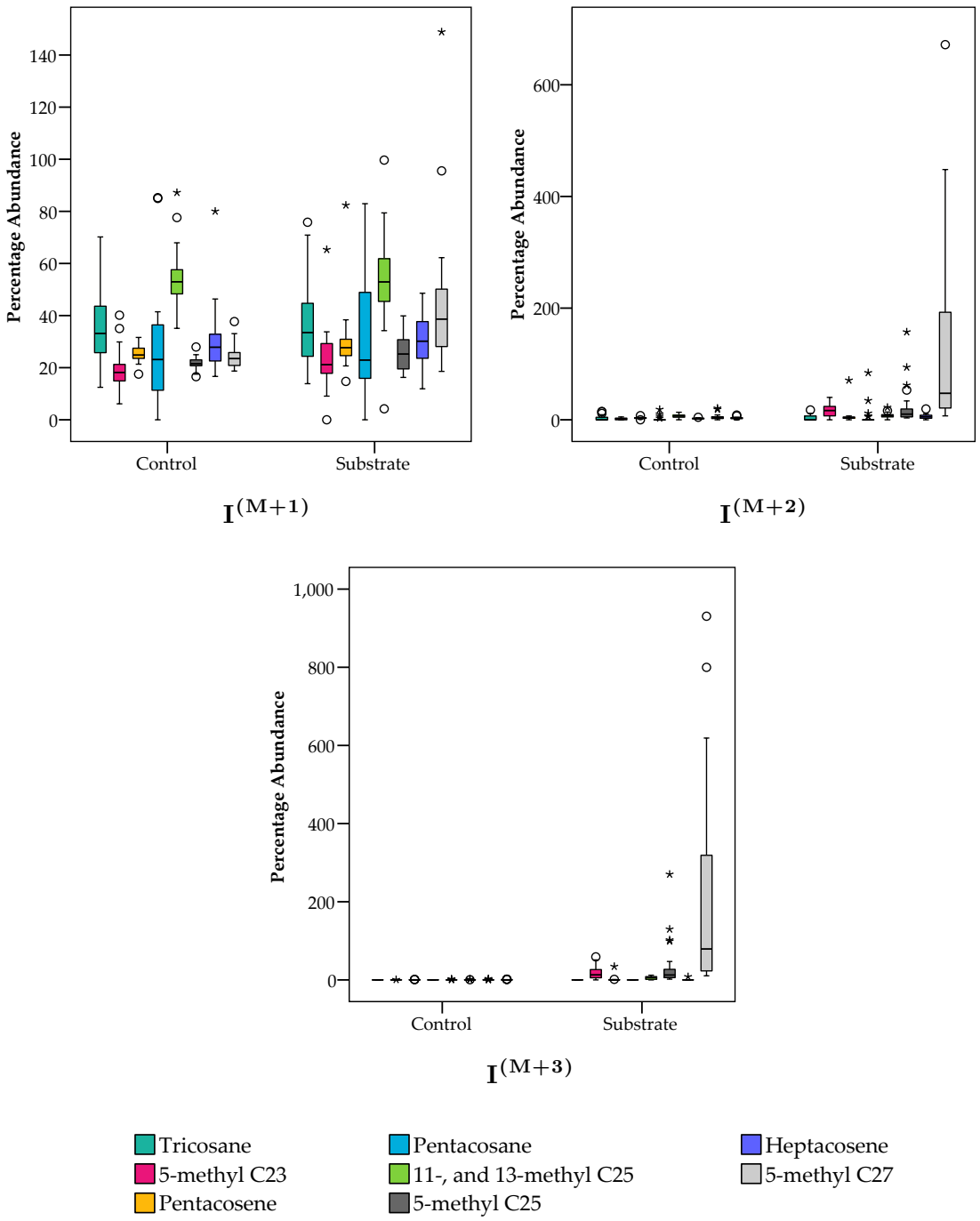


Figure 5.29: Boxplots showing the data for *Myrmica sabuleti* for DL-valine d_8. $I^{(M+1)}$ is on the left, $I^{(M+2)}$ on the right and $I^{(M+3)}$ below.

In the same way Table 5.6 shows the results of the Mann-Whitney statistical analysis of this data.

These results indicate that there is far more difference than originally apparent, see Figure 5.6. For $I^{(M+1)}$, $I^{(M+2)}$ and $I^{(M+3)}$ there is statistical difference between the con-

Table 5.6: The probability values from the calculated Mann-Whitney U-tests for *Myrmica sabuleti*, for DL-valine d_8, and all three isotope peaks. Note that any p values less than 0.050, and therefore significant, are underlined for ease of reference.

Compound	p value DL-valine d_8		
	$I^{(M+1)}$	$I^{(M+2)}$	$I^{(M+3)}$
Tricosane	0.368	0.493	1.000
5-methyl C23	<u>0.030</u>	<u>0.000</u>	<u>0.000</u>
Pentacosene	<u>0.012</u>	<u>0.026</u>	0.449
Pentacosane	0.222	0.227	1.00
11-, and 13-methyl C25	0.504	0.305	<u>0.000</u>
5-methyl C25	<u>0.009</u>	<u>0.000</u>	<u>0.000</u>
Heptacosene	0.086	0.081	0.214
5-methyl C27	<u>0.000</u>	<u>0.000</u>	<u>0.000</u>

trol group and the substrate group for the compounds 5-methyl C23, 5-methyl C25, and 5-methyl C27. This indicates that the deuterated valine is primarily incorporated into the 5-methyl-branched hydrocarbons, however other compounds at other isotope levels also show incorporation.

5.3.2.2 L-valine $^{13}C_5$,^{15}N

Formica lemani

Unfortunately at the end of this experiment only three live ants remained out of the sample group within the L-valine $^{13}C_5$,^{15}N substrate box. These were extracted and run as per the established procedure however the quality of this data was very poor and therefore not suitable for statistical analysis. However it is worth mentioning that despite the fact that these samples were not subjected to the normal statistical processing the raw mass spectra do not show any evidence of incorporation. This was crudely tested using the average percentage values for $I^{(M+1)}$ and $I^{(M+2)}$ for the pentacosene peaks. The average value of the pentacosene peak for $I^{(M+1)}$ and $I^{(M+2)}$ was taken from the three remaining L-valine $^{13}C_5$,^{15}N samples at the end of the experiment and

compared to equivalent average values from the L-leucine $^{13}C_6$,^{15}N experiment (substrate and control). The average percentage abudances for pentacosene were chosen on the basis that the intensity for the pentacosene peak was the most comparable across the datasets. Although an extremely basic test, the results are shown in Table 5.7.

Table 5.7: The $I^{(M+1)}$ and $I^{(M+2)}$ values for *Formica lemani* for the L-leucine $^{13}C_6$,^{15}N (labelled leucine), L-valine $^{13}C_5$,^{15}N (labelled valine) and control valine experiments. Values shown are average percentage abundance values for pentacosene.

Isotope peak	Experimental sample		
	Labelled leucine	Labelled valine	Control valine
$I^{(M+1)}$	37.2%	27.2%	26.7%
$I^{(M+2)}$	19.6%	4.3%	3.5%

As it can be seen the values for $I^{(M+1)}$ and $I^{(M+2)}$ are much greater in the sample that was from the L-leucine $^{13}C_6$,^{15}N experiment, whilst the values for $I^{(M+1)}$ and $I^{(M+2)}$ for the labelled valine experiment are more similar to those from the control. This difference is most noticeable at the $I^{(M+2)}$ level. This suggests that it is possible that these ants did not incorporate the labelled valine substrate into their hydrocarbons. However as the data is very poor this should only be taken as an indication of a possible result. A full analysis of this data can be found later on in this section.

Myrmica sabuleti

Again for this data it became apparent that large amounts of incorporation had occurred for this species and substrate. In addition the data indicated that incorporation of the substrate was widespread with a large number of extra isotopes present. Due to this the procedure for the data analysis for this species and substrate was adjusted and the values for $I^{(M+X)}$ were recorded up to, and including $I^{(M+15)}$. This was so that any trends within the data could be explored. However for the purposes of comparing this data against the control, only the peaks for $I^{(M+X)}$, where X=1-3, will be initially presented. Figure 5.30 shows the average ion counts for the compound pentacosene, it can be clearly seen that the amount and abundance of isotopes is far greater in the

substrate data set compared to the control. Note that this data only shows the comparative data for the compound pentacosene which showed maximum isotope levels of M+13.

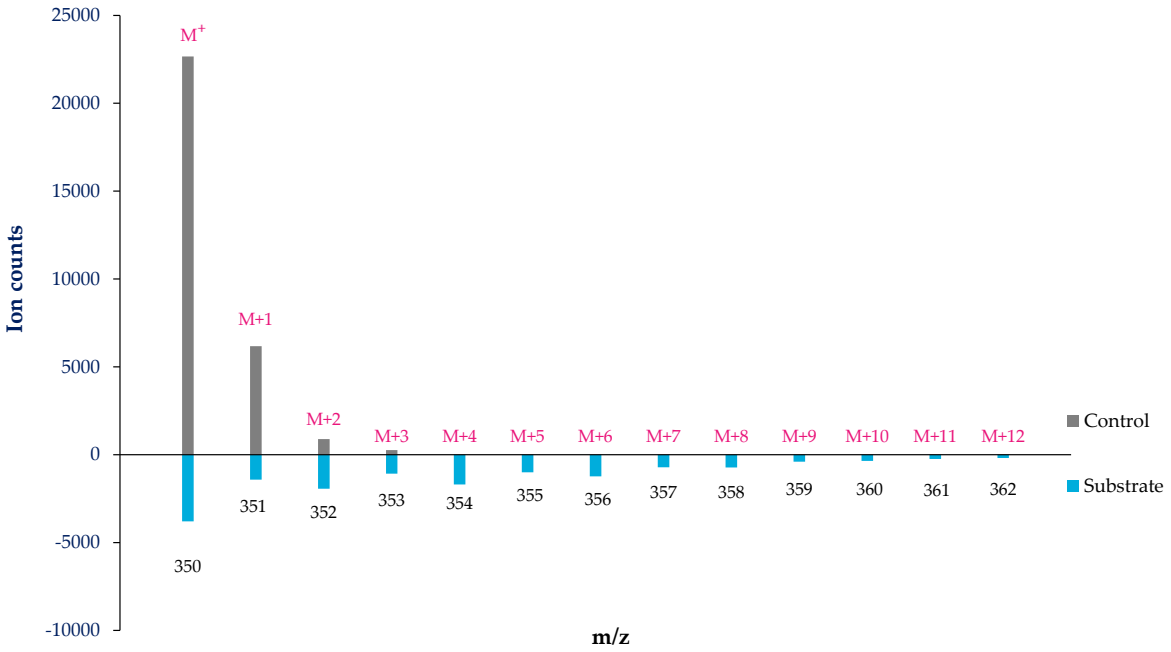


Figure 5.30: Extracted mass spectrum for *Myrmica sabuleti* and the L-valine $^{13}C_5$, ^{15}N substrate experiment showing average comparative ion counts for control and substrate data sets for the compound pentacosene.

It should also be noted that because this was a different colony to that used before the abundances of the compounds present within the chemical profile were slightly different. This meant that slightly different compounds were selected for analysis.

The data for *Myrmica sabuleti* and L-valine $^{13}C_5$, ^{15}N was generally fairly poor. This was thought to be mostly due to low instrumental sensitivity. Consequently the number of data files that were suitable for statistical analysis was much lower than desired. In addition there was no data available for pentacosane and therefore this compound was removed from analysis. For the statistical analysis n=6 for the substrate data and n=10 for the control data.

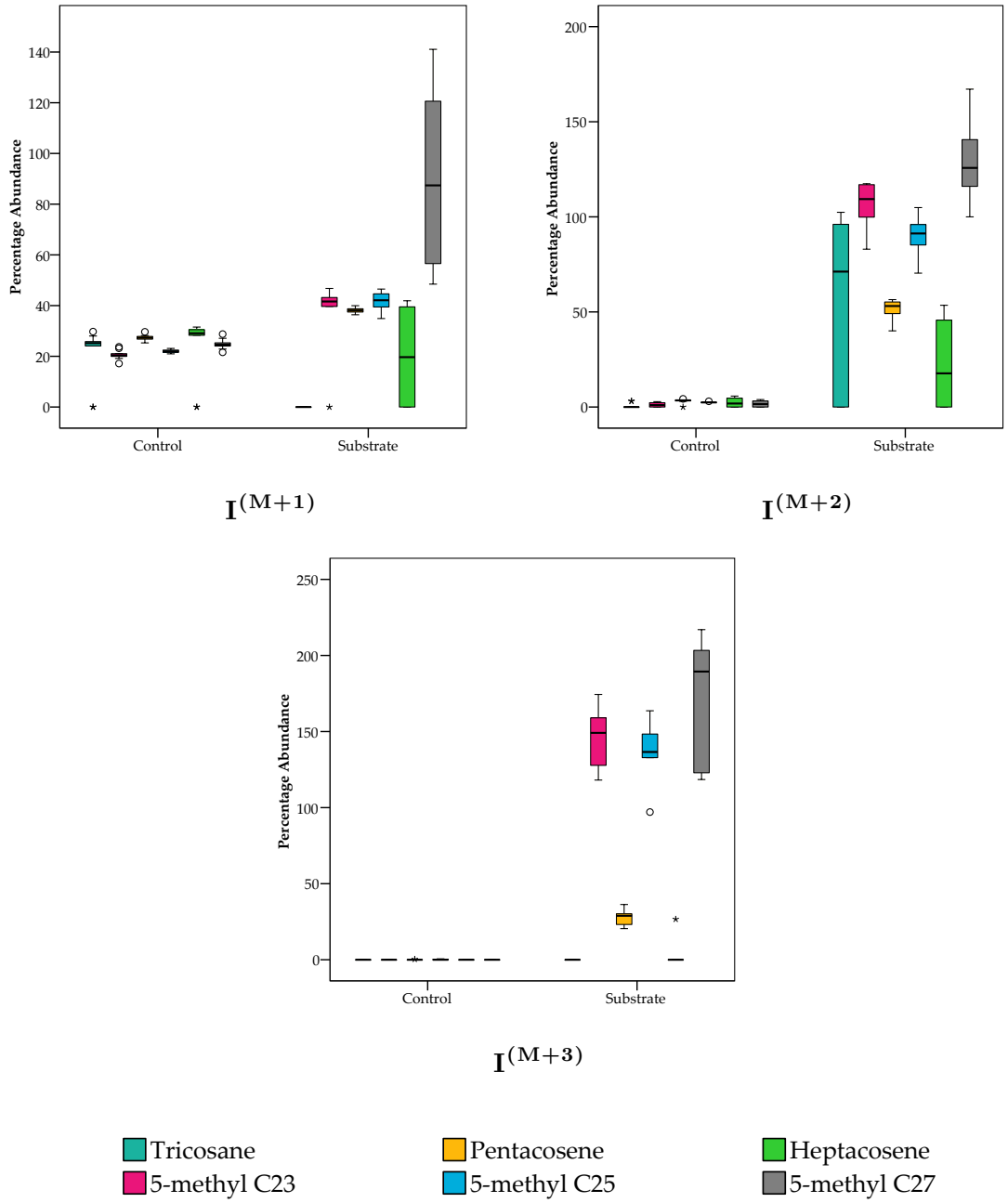


Figure 5.31: Boxplots showing the data for *Myrmica sabuleti* for L-valine $^{13}C_5$,^{15}N. $I^{(M+1)}$ is on the left, $I^{(M+2)}$ on the right and $I^{(M+3)}$ below.

As Figure 5.31 shows the data for the control samples was very consistent. There was much greater variation observed for the substrate samples. The greatest variation can be seen for the compounds heptacosene (green bar) and 5-methyl C27 (grey bar) for $I^{(M+1)}$, tricosane for $I^{(M+2)}$ (turquoise bar) and 5-methyl C27 for $I^{(M+3)}$. As can be seen from the boxplot data this variation is usually caused by some samples having values of 0.0% and some having much higher values. This suggests that once again

some samples within the data set show very strong incorporation whilst some show only marginal amounts. However this could also be down to instrument sensitivity and that although incorporation is occurring the intensity of the isotope peak is simply too small to measure.

Table 5.8 shows the Mann-Whitney U-test data for *Myrmica sabuleti* and L-valine $^{13}C_5$,^{15}N. As these values show there was no statistical difference between data sets for the compound heptacosene. A probability value of 1.000 can also be seen for tricosane for $I^{(M+3)}$. This is because for these samples all values were equal to 0.0% and therefore there was no measurable difference between the data sets for this compound at this level.

Table 5.8: The probability values from the calculated Mann-Whitney U-tests for *Myrmica sabuleti*, for L-valine $^{13}C_5$,^{15}N, and all three isotope peaks. Note that any p values less than 0.050, and therefore significant, are underlined for ease of reference. Any p values less than 0.050 which are italicised may be disregarded and are due to the median rank of the control being greater than that of the substrate group.

Compound	p value L-valine $^{13}C_5$,^{15}N		
	$I^{(M+1)}$	$I^{(M+2)}$	$I^{(M+3)}$
Tricosane	0.002	*0.026*	1.000
5-methyl C23	0.003	0.000	0.000
Pentacosene	0.000	0.000	0.000
5-methyl C25	0.000	0.000	0.000
Heptacosene	0.472	0.203	0.412
5-methyl C27	0.003	0.000	0.000

As previously mentioned the raw data files indicated that there was large amounts of incorporation occurring with the labelled substrate fed ants incorporating large amounts of extra mass into their biosynthesised hydrocarbons. Therefore for this substrate, and this species, data was recorded up to, and including $I^{(M+15)}$. This was because after $I^{(M+15)}$ the percentage abundances were approaching zero and therefore it was

felt that up to $I^{(M+15)}$ was an appropriate measurement. The graphs shown in Figure 5.32 represent the varying percentage abundances of the ion counts for the measured isotope peaks. The data is firstly shown as bar charts for the control and the substrate data. See Figure 5.32 for these graphs.

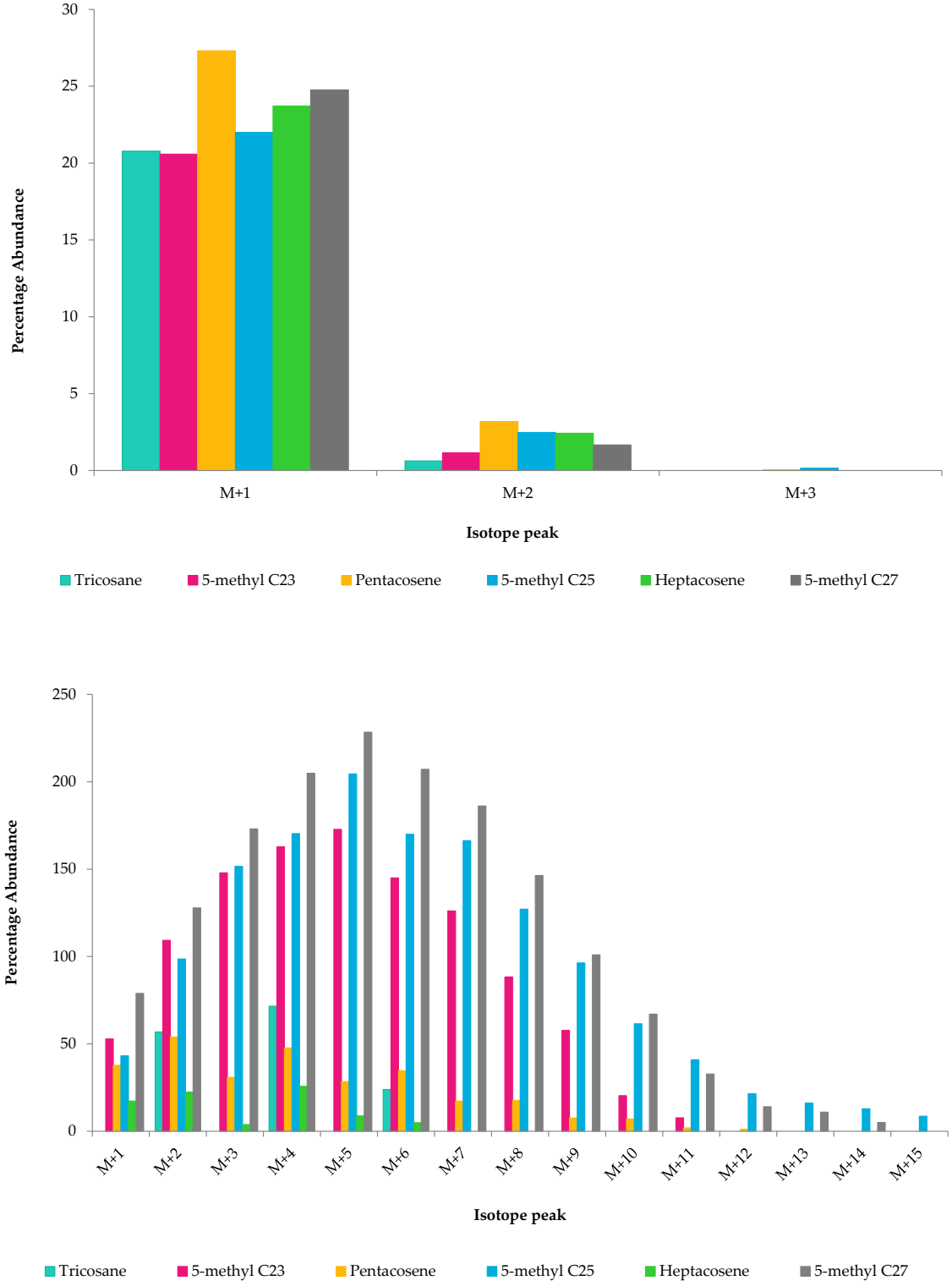


Figure 5.32: Bar chart showing the percentage abundance results of the DL-valine control data and the L-valine $^{13}C_5$,^{15}N substrate data for the studied isotope peaks for the species *Myrmica sabuleti*.

The difference in the two sets of data can be clearly seen. For the control data there were no $I^{(M+3)}$ peaks present with the exception of pentacosene and 5-methyl C25. The substrate data can generally be divided into two groups. The methyl-branched hydrocarbons follow the same trend in that all three compounds show a fairly steep increase until $I^{(M+5)}$. At this point all three compounds show a gradual downward trend. In contrast the three straight chain compounds do not show this trend. To explore these trends more thoroughly the substrate data was plotted as a line chart to show the individual compound trends more clearly. For this line chart see Figure 5.33.

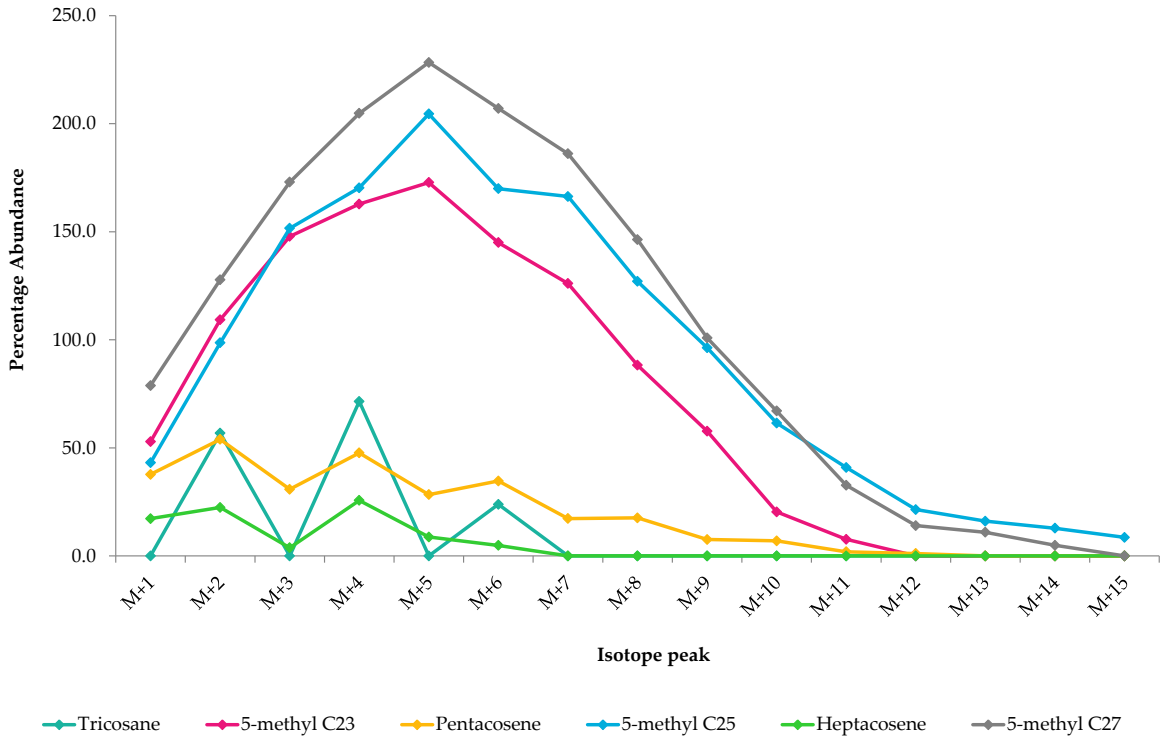


Figure 5.33: Line chart showing the downward trends in percentage abundances for each compound of the L-valine $^{13}C_5$,^{15}N substrate data for *Myrmica sabuleti.*

Figure 5.33 shows the difference more clearly between the straight chain and methyl-branched hydrocarbons. As the bar chart shows, the methyl-branched hydrocarbons show the greatest intensities. The straight chain hydrocarbons show a trend that is totally different to the methyl-branched hydrocarbons. These differences are highlighted by again splitting these compounds into groups based on types. For extra insight error

bars representing +/- two standard errors are added.

As mentioned the abundance of the compound pentacosane was too low to be recorded and as such it was removed from subsequent analysis. Consequently this meant that for this set of data there was only one alkane analysed. The results of this are presented in the line chart shown in Figure 5.34.

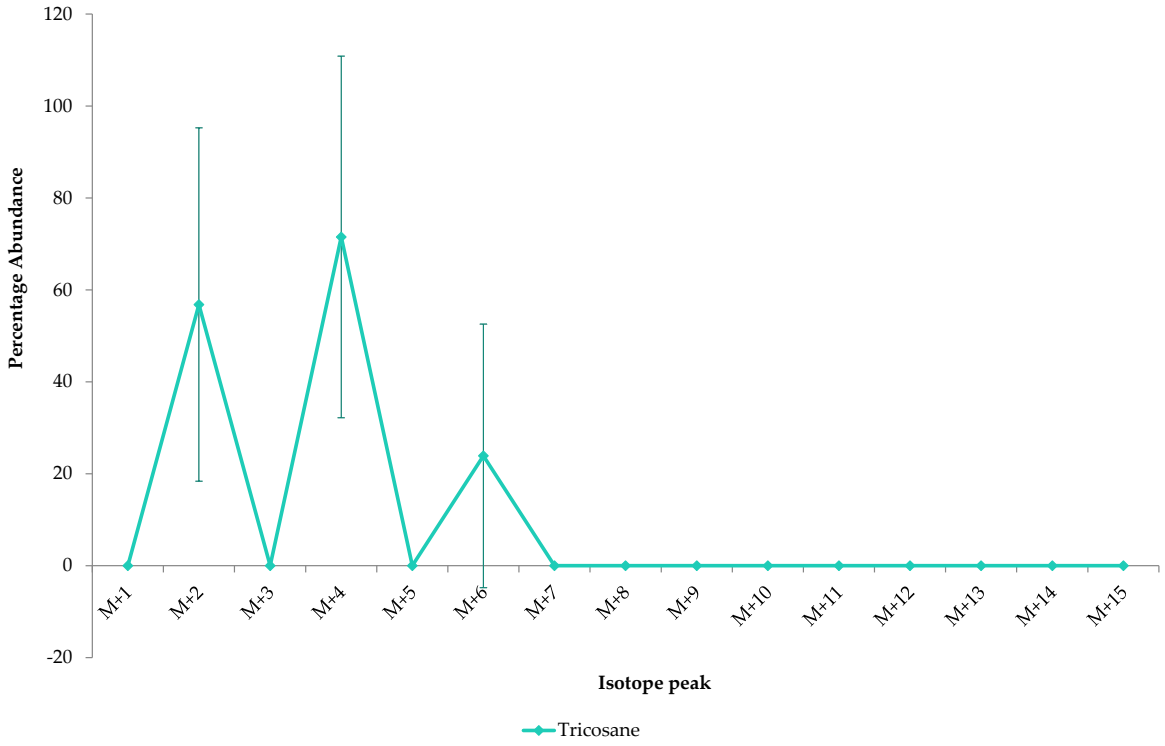


Figure 5.34: Line chart showing the downward trends in percentage abundances for tricosane for the L-valine $^{13}C_5$,^{15}N substrate data for *Myrmica sabuleti*.

It can be clearly seen that for this compound there is a significant bias towards the incorporation of even numbered amounts of mass and therefore percentage abundances are higher for even $I^{(M+X)}$ levels; where X=2, 4, and 6. However it should be noted that for this set of data the error bars are very large. Again this is due to the low quality of the data and sensitivity issues with the instrument.

In similar fashion this bias towards even units of mass incorporation is also seen for

Figure 5.35: Line chart showing the downward trends in percentage abundances for the alkenes of the L-valine $^{13}C_5$,^{15}N substrate data for *Myrmica sabuleti.*

the alkene compounds as shown in Figure 5.35. As a result the odd numbered $I^{(M+X)}$ values show very low percentage abundance values compared to those seen for the even values. Both alkene compounds (gold and green lines) show a gradual downward trend with fairly close correlation between the two.

Figure 5.36 shows just the methyl-branched compounds and it can be seen that these three compounds show a completely different trend to the straight chain hydrocarbons. The methyl-branched hydrocarbons all follow the same pattern in that there is a gradual increase until the peak maxima is reached; M+5 for all three compounds, followed by a gradual decrease. The rate of change for the increase is similar to that of the decrease until about M+10 whereby the rate of change becomes much more gradual until the values approach 0.0% at M+15. The bias towards even amounts of incorporation, as seen for the straight chain hydrocarbons, is not seen for the methyl-branched hydrocarbons at all. In fact, as previously mentioned, the maximum levels for all three

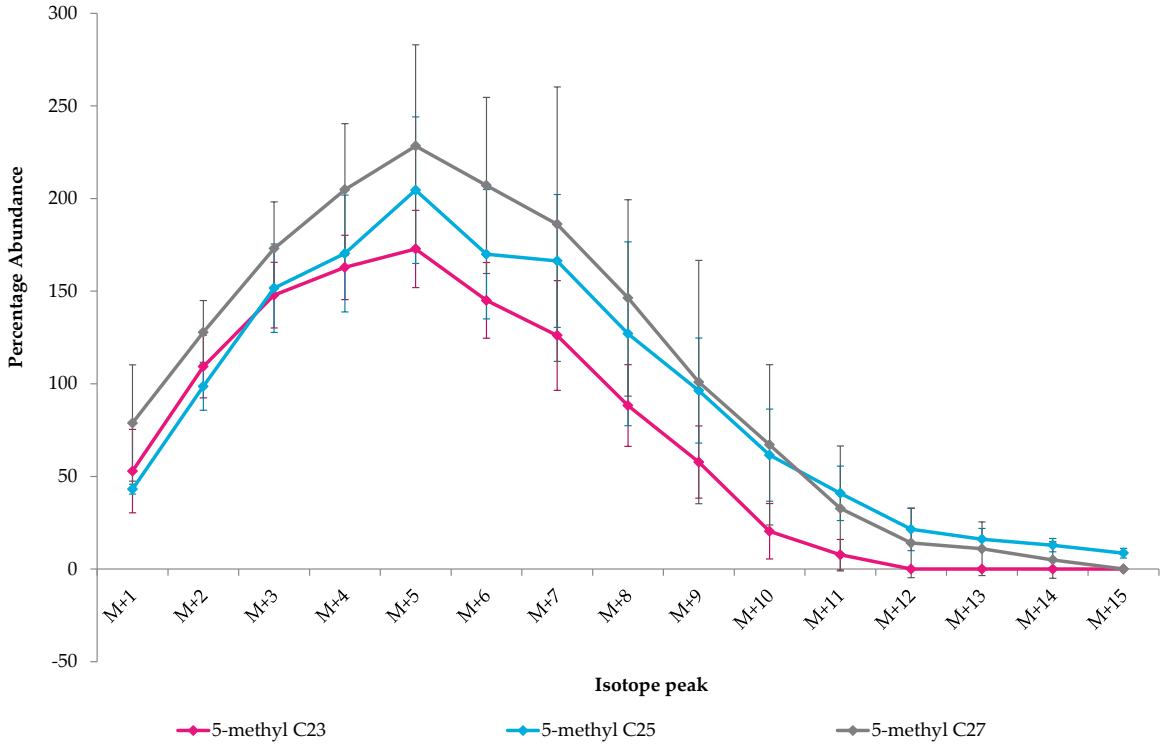


Figure 5.36: Line chart showing the downward trends in percentage abundances for the methyl-branched compounds of the L-valine $^{13}C_5$,^{15}N substrate data for *Myrmica sabuleti*.

of these hydrocarbons is observed for $I^{(M+5)}$, and not an even number. It may also be suggested that for 5-methylpentacosane (light blue) there may even be a bias towards odd units of mass incorporation. The differences seen in incorporation between straight chained and methyl-branched hydrocarbons suggests that there may be a difference in the way that this amino acid is broken down and subsequently used in the biosynthesis of these compound types. The bias towards even numbers of $I^{(M+X)}$ suggests that for straight chain compounds two, or even four mass units are added each time, whilst the maxima of $I^{(M+5)}$ for methyl-branched hydrocarbons suggests that maybe five units are added at a time. One of the ways further information can be gained on possible reaction mechanisms is to consider known biochemical reactions for L-valine $^{13}C_5$,^{15}N. These will be explored later on in the chapter.

5.3.2.3 Discussion

L-valine d_8

From the existing knowledge surrounding biosynthesis it is already known that the amino acids valine and leucine are key precursors in the formation of 2-methyl alkanes [2]. However neither *Formica lemani* or *Myrmica sabuleti* produce compounds of this type. In addition to being important for the synthesis of 2-methyl alkanes, valine can also be metabolised to form propionyl-CoA and from this methylmalonyl-CoA, which is used during the biosynthesis of internally branched hydrocarbons [2]. Other amino acids which can be metabolised to form propionyl-CoA are isoleucine and methionine [2]. Based on Table 5.6 it can be seen that the labelled substrate DL-valine d_8 is incorporated predominantly into the internally branched hydrocarbons of *Myrmica sabuleti*. From this it can be assumed that the mechanism behind this incorporation is likely to be one which is associated with the biosynthesis of methyl-branched hydrocarbons. Therefore this data suggests that valine is likely metabolised into methylmalonate, a molecule which is used in the biosynthesis of methyl-branched hydrocarbons, and hence shows clear incorporation for the methyl-branched hydrocarbons.

The metabolism of valine to form propionyl-CoA is one that is widely understood as it is used as a major pathway in the general breakdown of amino acids. Valine is first transaminated to the corresponding α-ketoacid, ketoisovalerate. This undergoes oxidative decarboxylation to yield isobutyryl-CoA. From this there is a sequence of four reactions; oxidation via FAD, hydration, oxidation and the final thiolysis step to yield propionyl-CoA and carbon dioxide [5, 9]. See Figure 5.37 for the full reaction scheme.

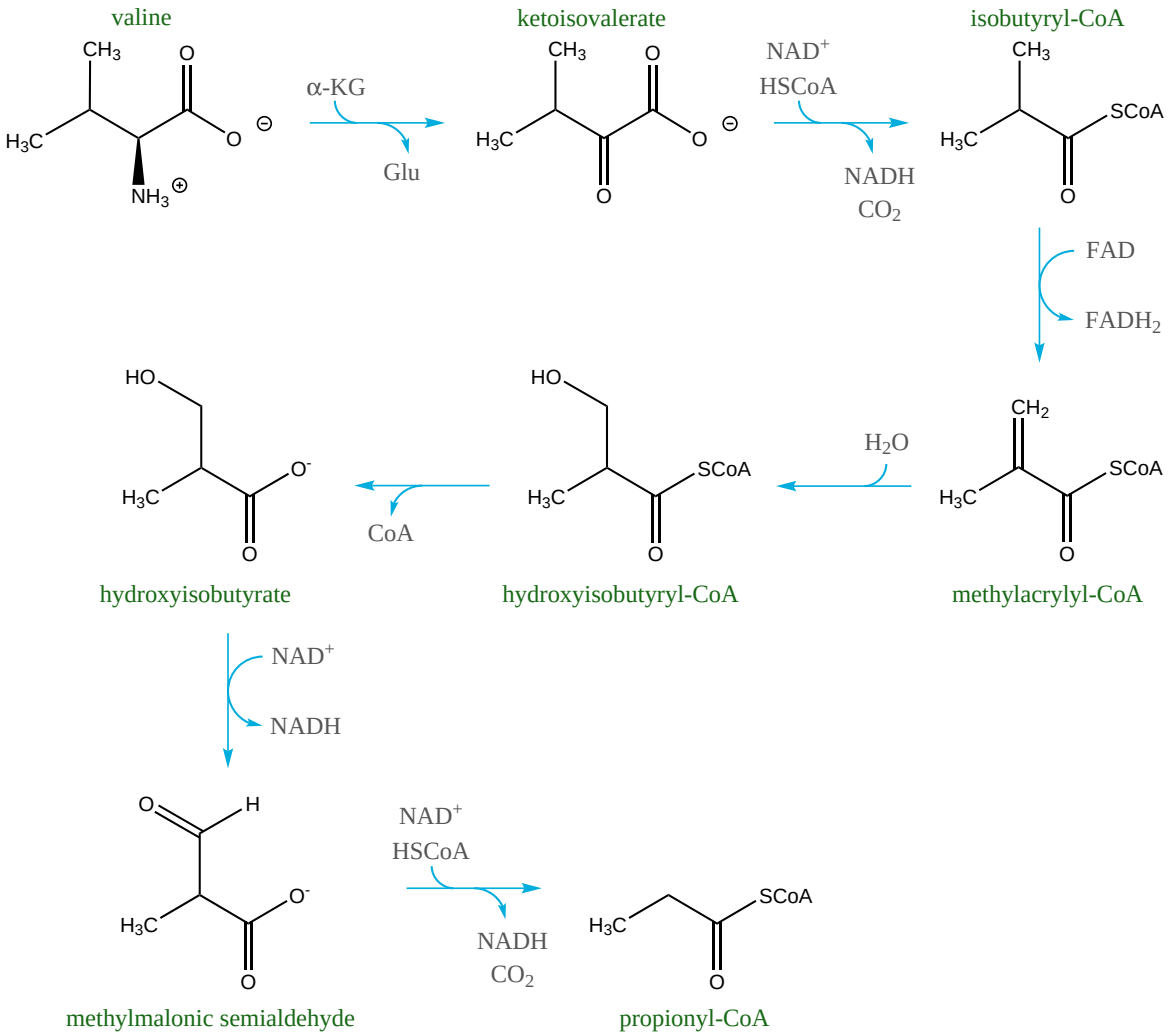


Figure 5.37: Reaction scheme showing the breakdown of valine to form carbon dioxide and propionyl-CoA, adapted from [5, 9].

Although the breakdown of valine suggests that propionyl-CoA is produced, the location of the resulting deuterium labels must be considered in order to determine whether this is the source of the deuterium atoms observed in the biosynthesised hydrocarbons.

For a reaction scheme which details the breakdown of DL-valine d_8 with all deuterium labels clearly shown see Figure 5.38. As Figure 5.38 indicates, for every molecule of DL-valine d_8 that is degraded, one molecule of labelled propionyl-CoA is produced which contains three deuterium labels on the terminal carbon. This propionyl molecule could theoretically be used in the biosynthesis of methyl-branched hydrocarbons, however it must not be assumed from this mechanism that the labels on the resulting

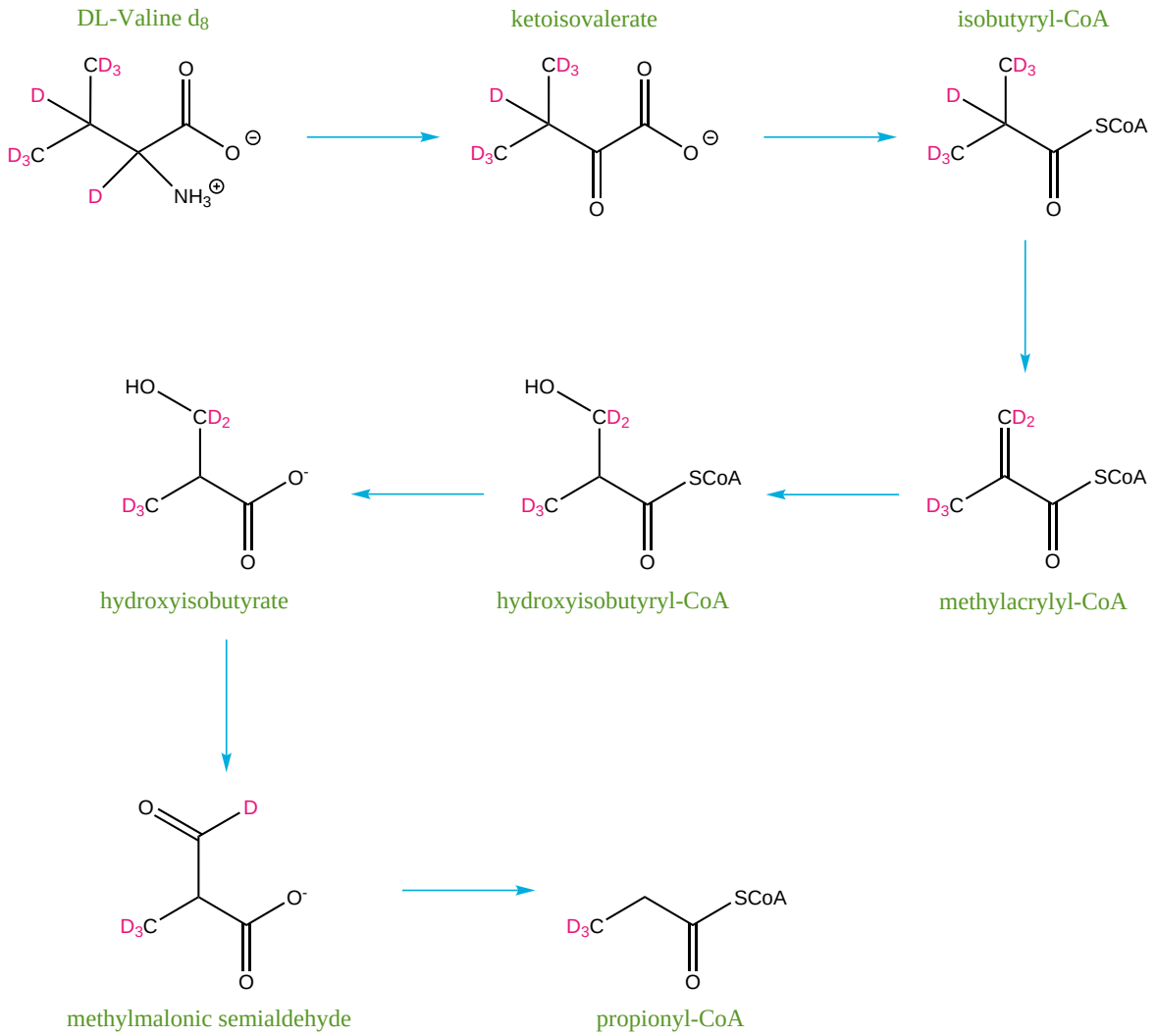


Figure 5.38: Reaction scheme showing the breakdown of DL-valine d_8 to form carbon dioxide and propionyl-CoA, adapted from [5, 9].

propionyl-CoA will survive intact into a biosynthesised cuticular hydrocarbon. In order to ascertain whether these deuterium labels would be incorporated, the mechanism for the incorporation of propionyl-CoA into methyl-branched hydrocarbons must be considered, see Figure 5.39. It should be noted however that the final product shown in this reaction scheme would then be subject to the elongation steps shown in Figure 1.14 in order to yield the final hydrocarbon product.

As Figure 5.39 shows, when this labelled propionyl is used to biosynthesise a methyl-branched hydrocarbon, the three deuterium atoms end up on the carbon of the methyl

branch. This suggests that each resulting labelled methyl-branched hydrocarbon will be three mass units heavier. However it is well known that deuterium atoms will readily exchange with hydrogen, this may explain why various mass differences are seen i.e. for *Myrmica sabuleti* all three $I^{(M+n)}$ levels show statistical difference for all three of the 5-methyl compounds. Given that each labelled valine incorporated should add 3 Da to the final branched hydrocarbon it would not be expected that $I^{(M+1)}$ and $I^{(M+2)}$ would also show such a low p value, however if we consider hydrogen exchange then this becomes more understandable.

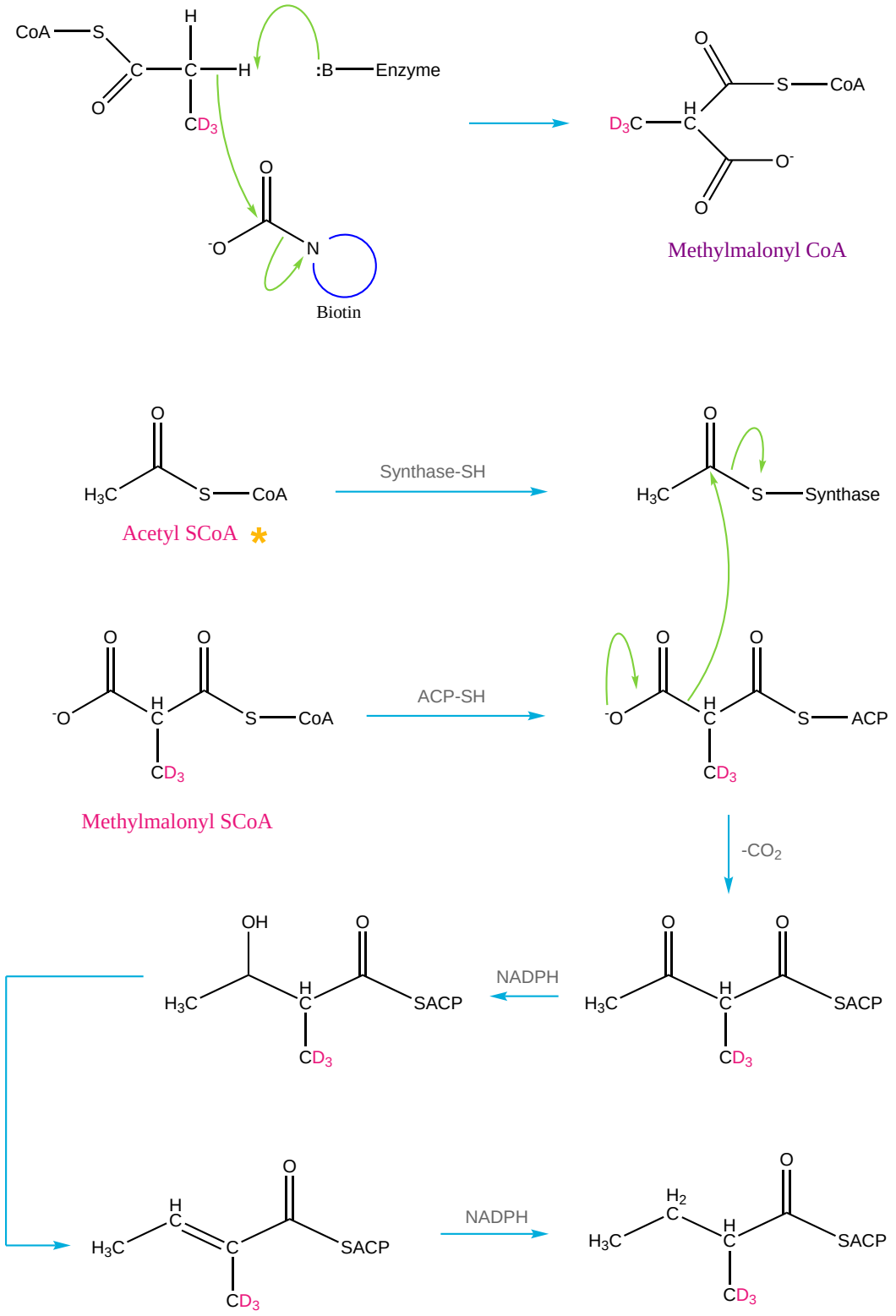


Figure 5.39: Reaction scheme for the incorporation of the labelled propionyl-CoA into a methyl-branched hydrocarbon.

Table 5.6 also indicates that there is statistical significance for the straight chained compound pentacosane at the $I^{(M+1)}$ and $I^{(M+2)}$ level. The incorporation seen for this

compound cannot be due to the mechanism previously discussed as this is for methyl-branched compounds only. It may therefore be assumed that this can be explained via the knowledge that these species can metabolise propionate into acetate which can then be used in the biosynthesis of straight chain cuticular hydrocarbons. However when following this mechanism through it can be seen that according to the pathway suggested by Halarnkar [10] the deuterium labels would be lost from the acetate molecule produced. However as Table 5.6 shows there is significant incorporation of a label for pentacosane at the $I^{(M+1)}$ and $I^{(M+2)}$ level, therefore this isotope must be originating from somewhere. All of the metabolic reactions described are facilitated by complex molecules such as FAD and NADP. These molecules can accept hydrogen to become reduced, or donate them and become oxidized. It is possible therefore that these molecules accept deuterium atoms as they would hydrogens and in turn these are donated back later on, either in the same reaction or a different one. This may explain why a small amount of labelling is seen for pentacosane.

L-valine $^{13}C_5$,^{15}N

In contrast to the data observed for DL-valine d_8, which predominantly suggested incorporation for the internally branched hydrocarbons, the data for L-valine $^{13}C_5$,^{15}N suggested much more widespread incorporation. However given that the species data for this substrate was so poor for *Formica lemani*, this species will not be analysed further. Instead the analysis of the data will focus on that obtained for *Myrmica sabuleti*. This data showed widespread incorporation for the methyl-branched and straight chain hydrocarbons with subtle differences observed for the two compound types. The straight chain hydrocarbons showed a bias toward the incorporation of even numbers of extra mass, whilst the methyl-branched hydrocarbons indicated that the maximum isotope intensity was seen for M+5. Therefore this suggests that there are potentially two different mechanisms of incorporation in action; one for the methyl hydrocarbons and one for the straight chain hydrocarbons. As Figure 5.37 suggests the metabolic breakdown of valine produces propionyl-CoA and carbon dioxide [5, 9]. However once

again the position of the labelled carbon atoms for L-valine $^{13}C_5$,^{15}N within this reaction scheme must be considered. See Figure 5.40 for this mechanism with the position of the carbon-13 atoms clearly indicated.

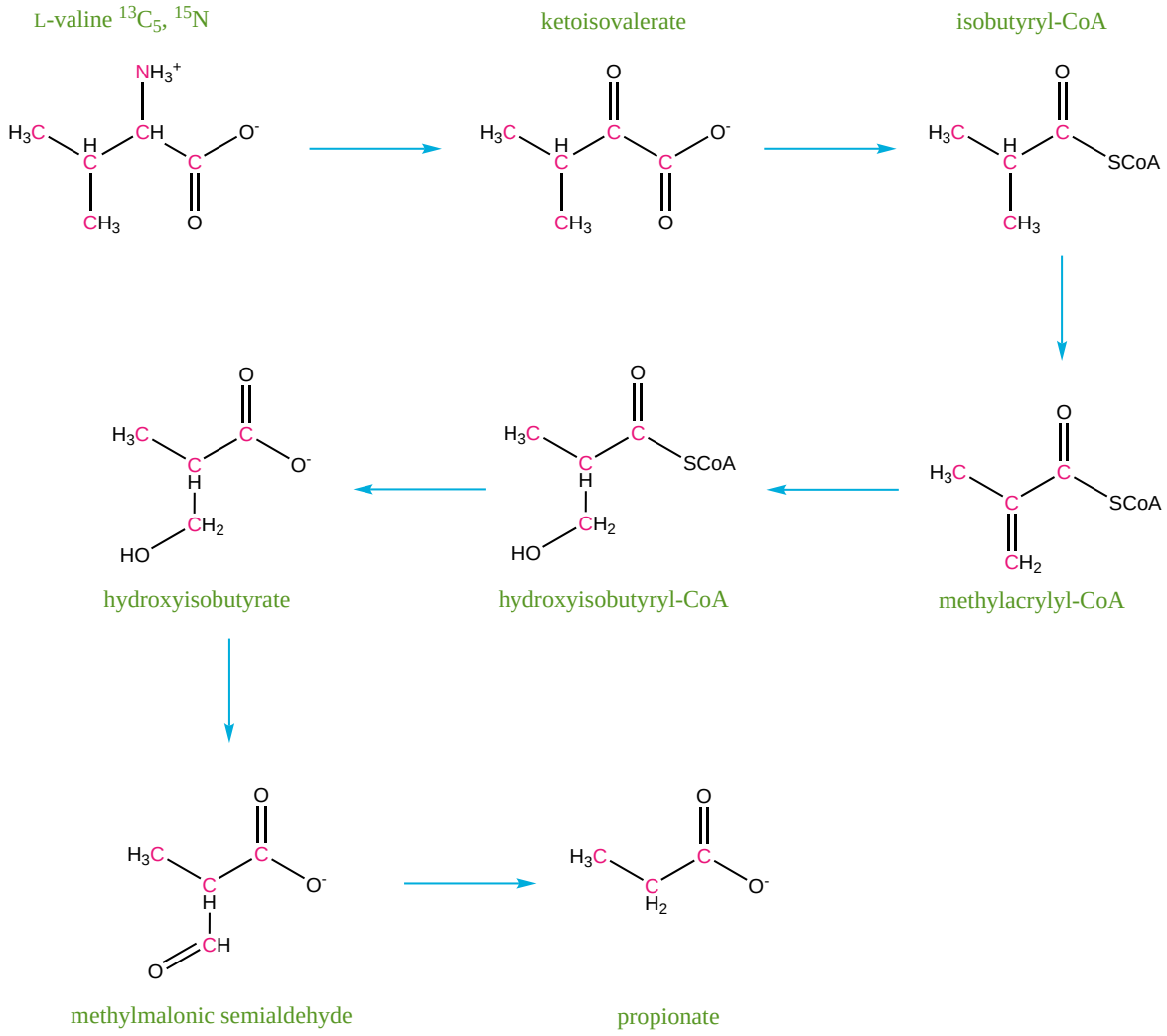


Figure 5.40: Reaction scheme showing the breakdown of L-valine $^{13}C_5$,^{15}N to form carbon dioxide and propionyl-CoA, adapted from [5, 9].

As the mechanism shown in Figure 5.40 indicates, the product of the breakdown of L-valine $^{13}C_5$,^{15}N is a propionyl-CoA molecule with three carbon-13 atoms. As previously mentioned propionyl itself is a very biologically important molecule in the synthesis of cuticular hydrocarbons. It can primarily be used in the formation of methyl-branched hydrocarbons. However once again it cannot be assumed that all three of these labelled atoms will be subsequently incorporated into the biosynthe-

sised methyl-branched hydrocarbon. In order to ascertain what labels are ultimately incorporated, the mechanism for the use of this labelled propionyl to biosynthesise a methyl-branched hydrocarbon must be considered.

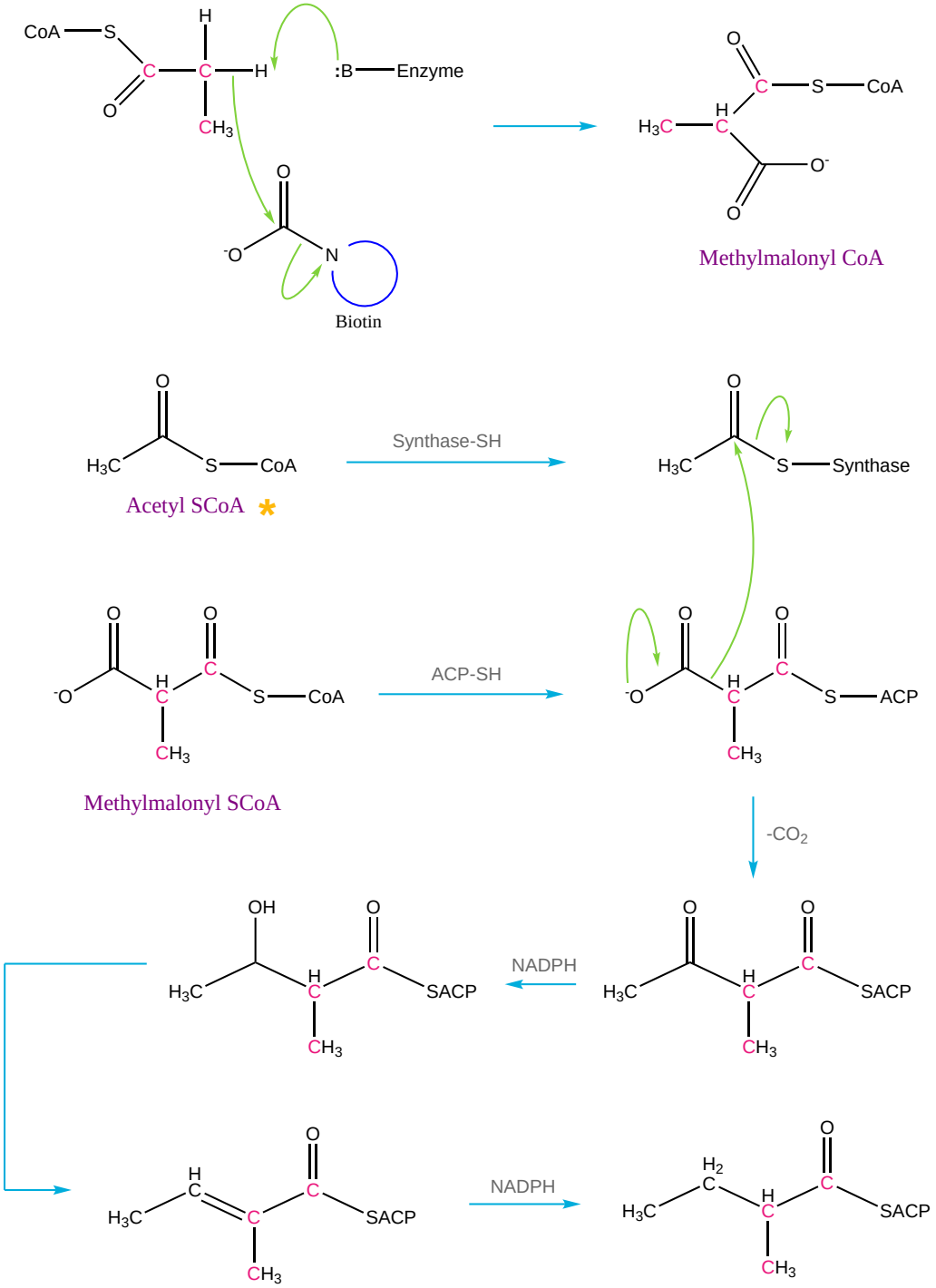


Figure 5.41: Reaction scheme for the incorporation of the labelled propionyl-CoA into a methyl-branched hydrocarbon showing how three extra mass units are incorporated.

The mechanism presented in Figure 5.41 show that it is possible that three extra units are incorporated into the methyl-branched hydrocarbons. However as the results indicated, the incorporation of five extra mass units was the most abundant for each of the methyl-branched hydrocarbons, suggesting that there must another process which is adding additional mass to these branched hydrocarbons. In Chapter 4, Section 4.3.3, the hypothesised metabolism of propionate to form acetate was discussed [10]. This is a relatively simple breakdown of the propionate to yield acetate, however again, in order to know which labels and how many survive this process, this breakdown must be considered based on the labelled propionate molecule from Figure 5.40.

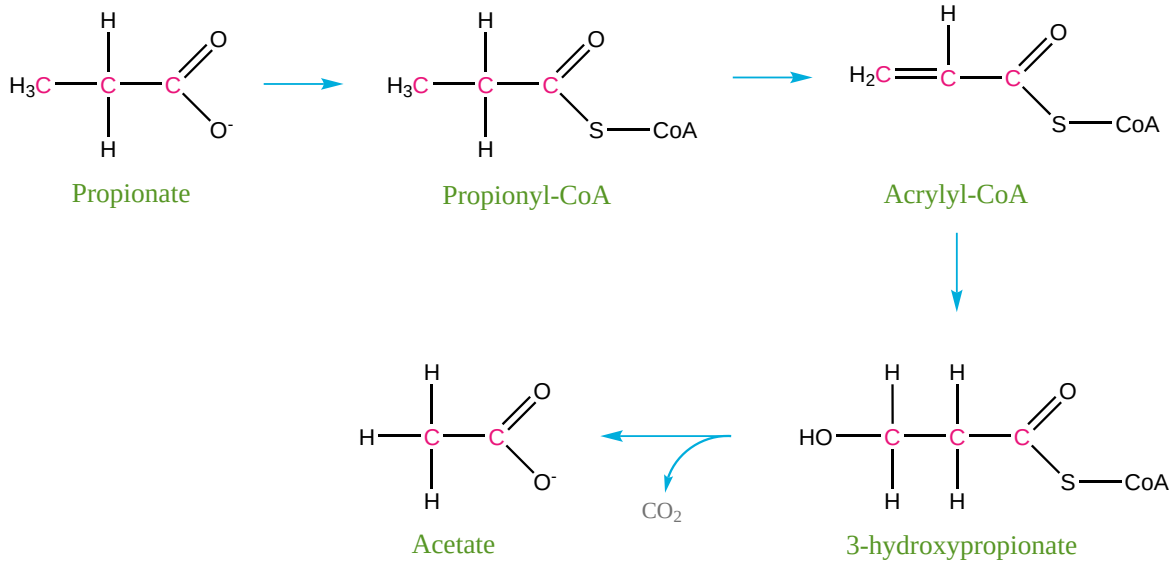


Figure 5.42: Schematic showing the hypothesised metabolism of labelled propionate into acetate, in this scheme the carbon-13 labels are indicated. Adapted from [10].

As this breakdown shows, for every labelled molecule of propionate that results from the initial breakdown of labelled valine, an acetate molecule is ultimately formed that contains two labelled carbon atoms. Acetate is a very biologically important molecule in terms of cuticular hydrocarbon biosynthesis as it is used for a variety of different purposes. Acetate can be used both in the *de novo* biosynthesis of fatty acids, and during the elongation of precursor fatty acids throughout hydrocarbon biosynthesis. As such acetate can be used during the biosynthesis of methyl-branched hydrocarbons

and straight chain hydrocarbons (both saturated and unsaturated). As Section 4.3.3 described, a methyl-branched hydrocarbon is biosynthesised via the insertion of a propionate molecule (at the right time) during the elongation of the fatty acid chain. As Figure 5.41 outlined this could potentially introduce three extra mass units into the methyl-branched hydrocarbon. However, as alluded to above, if some of the labelled propionate is also broken down to subsequently labelled acetate then this could also be introduced into the biosynthesis. In order to see what the resulting label incorporation would be in this situation, the mechanism for the incorporation of one unit of labelled propionate and one unit of labelled acetate will be presented. As Figure 5.41 indicated one molecule of methyl malonate is used to insert the methyl-branch into the growing hydrocarbon and produces the labelled fatty acid product seen in this reaction mechanism. If this chain elongation is continued with labelled acetate, it can be seen that a hydrocarbon with five extra mass units is possible. For this reaction scheme see Figure 5.43. This Figure only shows a basic reaction scheme indicating the steps rather than a full mechanism. A more detailed mechanism is presented later in this section.

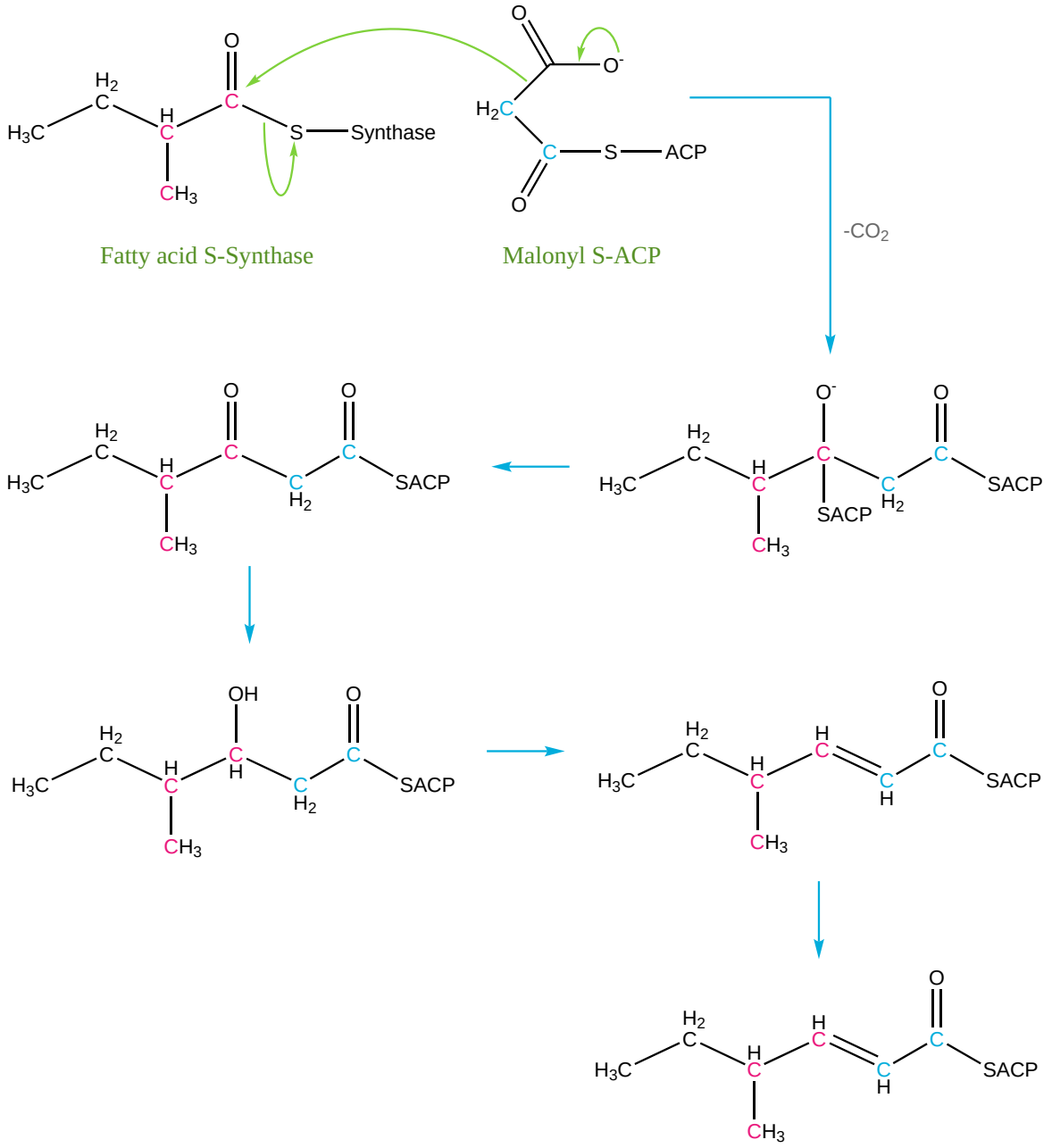


Figure 5.43: Reaction scheme showing the incorporation of labelled malonate, (shown in blue), into a labelled fatty acid precursor, (shown in pink) and the subsequent chain elongation steps. Note that the fatty acid precursor is that obtained from Figure 5.41 and the labelled acetate is that obtained from Figure 5.42, which is subsequently converted into labelled malonate prior to use.

As these Figures show, via this series of reactions it is possible to produce a methyl-branched hydrocarbon with five extra mass units incorporated. Three of these units originate from the breakdown of the labelled valine into labelled propionate, which is

then utilised as labelled methyl malonate to insert the branch. The additional two mass units originate from the further metabolic breakdown of labelled propionate into labelled acetate, this is then utilised as labelled malonate. Whilst these schemes only show the incorporation of one labelled malonate it must be remembered that a fatty acid will undergo many of these elongation reaction cycles to become the desired chain length, therefore it is possible that multiple labelled acetates could be incorporated in this way. This would give rise to the M+7, and M+9 isotope peaks seen. It should also be remembered however that if the last elongation cycle involves a labelled acetate then one of these labelled carbons will subsequently be removed when the fatty acid is then converted into the respective alkane. This explains the potential origins of the even mass numbers within the methyl-branched hydrocarbons.

The metabolic breakdown of propionate into acetate may also be key to the incorporation of extra mass into the straight chain hydrocarbons. As the results for this showed there was a clear bias towards the incorporation of even numbers of extra mass i.e. M+X where X=2, 4, 6 etc. Acetate is a biologically important molecule for the production of these hydrocarbons as it can be used either to elongate a fatty acid (first converted into malonate) or in the *de novo* biosynthesis of a fatty acid. However if acetate is used during the *de novo* biosynthesis of a fatty acid then it should be remembered that only the initial step involves acetate; the subsequent elongation of the chain utilises acetate in the form of malonate. The reaction mechanism shown in Figure 5.44 demonstrates how the labelled acetate produced from the breakdown of labelled valine (via propionate), can be used to biosynthesise a *de novo* fatty acid.

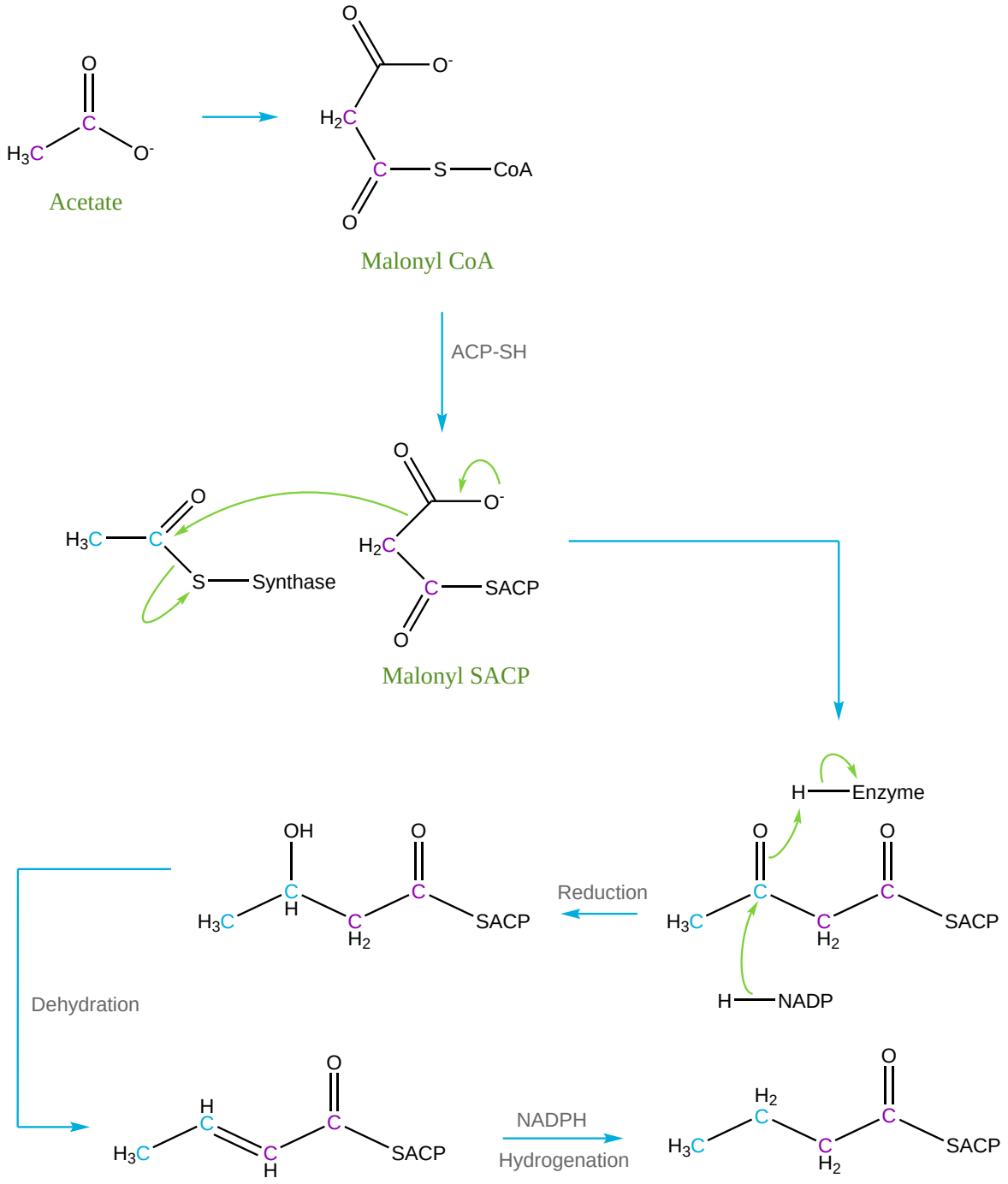


Figure 5.44: Schematic showing the incorporation of labelled acetate (shown in blue) and labelled malonate (shown in purple) into cuticular hydrocarbons. Note that the both the labelled acetate and the labelled malonate are derived from the degradation of labelled propionate shown in 5.42.

Within this process there are many steps; the labelled valine is broken down into labelled propionate and then into acetate, also isotopically labelled. Generally, in order

to elongate a fatty chain, acetate is used in the form of malonate. If the acetate used to make the malonate is labelled then the resulting malonate molecule will also be labelled. In Figure 5.44 this process, and resulting labelled malonate-CoA, is shown with carbon-13 atoms indicated in purple. However it should be remembered that the first step of *de novo* fatty acid biosynthesis involves a reaction between one molecule of malonate and one molecule of acetate. It is entirely possible that this acetate molecule is also labelled as it too originated from a molecule of labelled valine (via the labelled propionate intermediate). In Figure 5.44 this acetate is shown with carbon-13 labels in blue. As this mechanism shows, both of these processes yield a fatty acid that has two extra mass units incorporated for each process. There are several combinations of this process involving labelled and unlabelled acetates, however each results in an extra two mass units.

Whilst the final step of the biosynthesis of a straight chain hydrocarbon involves the loss of one of the terminal carbons to form an alkane it is more likely that this will be unlabelled than labelled. It is these possible mechanisms that may explain the bias towards even numbers of incorporation within the straight chain hydrocarbons.

5.4 Conclusions

The purpose of this chapter was to test our study species with substrates consisting of the labelled amino acids valine and leucine. The results of the substrate experiment using *Formica lemani* and L-leucine 5,5,5-d_3 indicated that there was incorporation of the deuterium isotopes into a variety of straight chain compounds but only at the $I^{(M+2)}$ level. This finding suggested that there may be a process which involved the addition of two deuteriums within the formation of the straight chain hydrocarbons. However based on assumed mechanisms and the known position of the deuterium labels no link could be found between the mass increase of two units and the predicted pathways. Instead it is suggested that the reason for finding extra mass within the straight chain hydrocarbons is as a result of the leucine breakdown to form acetoacetate, which can be further broken down to form acetate, or acetyl-CoA. This can then be used in the formation of straight chain hydrocarbons as previously discussed. Further evidence for this being the case can be seen with the results for *Myrmica sabuleti*, this time pentacosene showed the label, but at the $I^{(M+1)}$ level. The random nature of the incorporation suggests that it is a minor pathway that is responsible, and the different mass increases support the theory that it is a labelled acetate behind it as numerous acetate molecules can be used per one molecule of straight chain hydrocarbon produced.

The results for L-leucine $^{13}C_6$,^{15}N were much clearer with both species showing large amounts of incorporation. Isotope peaks up to and including M+10 for *F. lemani* and M+15 for *M. sabuleti* were recorded showing the extent of the incorporation. Each species showed a different overall trend however with *F. lemani* showing a gradual decrease and *M. sabuleti* an increase and then decrease. The reasons for this are currently unknown but it is suspected that this is a general difference caused by the amount of food consumed. The explanation for the random incorporation seen for L-leucine 5,5,5-d_3 was hypothesised to be due to the metabolic breakdown of leucine to form acetyl-CoA which can then be used to biosynthesise many different hydrocarbons.

The results from the experiment with L-leucine $^{13}C_6$,^{15}N again suggest this to be the case. Unlike with L-leucine 5,5,5-d_3 the initial breakdown of L-leucine $^{13}C_6$,^{15}N produces a labelled acetyl-CoA as well as further labelled acetyl-CoA via the subsequent breakdown of acetoacetate. It should also be considered that the non-labile nature of the carbon label could also be why this incorporation is much stronger. Finally when comparing the results from these experiments it must also be remembered that the differences in intensity could be down to other factors such as the age, and health of the ants used for this experiment.

To conclude, the results from these labelled leucine experiments show that leucine could be an important biological precursor to the biosynthesis of cuticular hydrocarbons, for both ant species, via a complex series of metabolic breakdowns to ultimately yield acetyl-CoA molecules. This suggests that there is an inherent flexibility with regards to the source of these molecules and despite the fact that, according to the literature, leucine was not previously thought to play a large role in hydrocarbon biosynthesis, this is not necessarily the case, with these results suggesting that leucine can be widely used.

In contrast, based on the existing literature it was expected that there would be clear incorporation of the DL-valine d_8 substrate as it is known that the degradation pathway of valine produces propionate which is then converted to methylmalonate and used in the biosynthesis of branched cuticular hydrocarbons. The results of the substrate experiment which utilised DL-valine d_8 supported this theory as the extra mass from the incorporated isotopes was found predominantly within the methyl-branched hydrocarbons. There were differences between the two species however. The results for *Formica lemani* showed that extra mass was detected only on the branched hydrocarbons and this extra mass was generally three units. In contrast, for *Myrmica sabuleti*, incorporation into the methyl-branched hydrocarbons was found at all three $I^{(M+n)}$ levels, it was thought that this may be due to exchange of the deuterium labels for hydrogen atoms, resulting in varied amounts of extra mass. From this it was shown to be likely that

Formica lemani and *Myrmica sabuleti* are able to metabolise valine into propionate, which supports previous research. This incorporation was very clear; the extra mass could be seen simply by studying the mass spectra produced.

There was however additional incorporation seen for *Myrmica sabuleti*. The results of this species, when fed DL-valine d_8, showed extra mass in the straight chain hydrocarbon pentacosene. This was a surprising result, at first it was assumed that this was a result of propionate metabolism to form acetate molecules which can then be used in the biosynthesis of straight chain compounds. However when these mechanisms were considered with the labelled molecules it was found that all three deuterium labels found on the propionate molecule, obtained through the degradation of valine, were lost when this propionate was converted to acetate. Therefore this could not be the source of the extra mass seen. Although the reasons for this observation are not fully understood, one theory is that the molecules FAD and NADP can act as acceptors and donors of hydrogen atoms, or in this case deuterium atoms, and thus the labels may end up on straight chain molecules or indeed any biosynthesised compound without there necessarily being a clear pathway.

Unfortunately no data was possible for the experiment with L-valine $^{13}C_5$,^{15}N and *Formica lemani*. However the data for L-valine $^{13}C_5$,^{15}N and *Myrmica sabuleti* showed much clearer incorporation. These results showed the same trends as those seen for DL-valine d_8 and *F. lemani* and *M. sabuleti* in that, on the whole, only the methyl-branched hydrocarbons showed incorporation. As before the possible hypothesis for this was that the valine is metabolised to form propionate which is then predominantly incorporated during the biosynthesis of methyl-branched hydrocarbons. Given that both of these labelled substrates have indicated this mechanism it is likely that this is the biosynthetic pathway. However it should also be noted that incorporation of labelled valine was not totally restricted to the methyl-branched hydrocarbons with some straight chain hydrocarbons showing incorporation. Again the explanation as to

why this is seen for L-valine $^{13}C_5$,^{15}N is likely the same as that for DL-valine d_8 in that valine can be metabolised primarily to form propionate, which in turn can be used for methyl-branched hydrocarbons. However the additional incorporation seen for both valine substrates lends further evidence that this propionate can also be metabolised further to form labelled acetate, and from this be used to biosynthesise a large variety of hydrocarbons.

The literature suggested that it would be likely that valine would be incorporated as it is known that this can be metabolised to form propionate based on previous experiments. However it is important that these assumptions are tested with a variety of different species. Based on the results of these experiments with labelled valine it can be seen that it is highly likely that *Myrmica sabuleti* can metabolise valine to propionate as suggested in the literature. It is also highly likely that the resulting propionate can be further broken down into acetate and from here used to biosynthesise a variety of compounds.

As previously discussed one of the main issues with the use of the deuterium labelled amino acids was that the labels were hydrogen based, not carbon. There are two issues with the use of deuterium labels. The first is that the labile nature of deuterium atoms means that they can be easily lost throughout the complex changes which these molecules undergo, whereas carbons are not so easily exchanged. This can give confusing results, which lack any clear trend. The second issue is that the resulting biosynthesised hydrocarbons cannot be further analysed using proton NMR in order to determine the position of the final label. Originally budgetary constraints made the use of carbon-13 labels impossible, but given the sporadic nature of the results obtained for the cheaper, deuterated substrates, it was felt that the extra cost was justified. The subsequent use of the carbon-13 substrates was extremely useful as they provided extra evidence for previously hypothesised biosynthetic pathways.

References

[1] Sadava D, Heller HC, Orians GH, Purves WK, and Hillis DM, *Life: The Science of Biology, 8th Edition*, (Sinauer 2008)

[2] Blomquist GJ, *Insect Hydrocarbons*, (Cambridge University Press, 2010)

[3] Martin S, and Drijfhout F, 'A review of ant cuticular hydrocarbons,' (2009), *J. Chem. Ecol.*, 35(10), 1151-1161

[4] Morgan ED, *Biosynthesis in Insects*, (Royal Society of Chemistry, 2010)

[5] Berg J, Tymoczko J, and Stryer L, *Biochemistry 5th Edition*, (Freeman, 2002)

[6] Anon., Department of Chemistry, University of Guelph http://www.chembio.uoguelph.ca/educmat/chm452/lectur26.htm (last accessed April 2015)

[7] Candy DJ, and Kilby BA, *Insect Biochemistry and Function*, (Springer, 1975)

[8] Willmer P, Stone G, and Johnston I, *Environmental Physiology of Animals*, (Blackwell, 2005)

[9] Anon., Department of Chemistry, University of Guelph http://www.chembio.uoguelph.ca/educmat/chm452/answers7.htm (last accessed April 2015)

[10] Halarnkar PP, and Blomquist GJ, 'Comparative aspects of propionate metabolism,' (1989), *Comp. Biochem. Physiol.*, 92B(2), 227-231

Chapter 6

Labelled substrate feeding experiment: fatty acids

6.1 Introduction

Fatty acids are compounds which contain a carboxylic acid group and a long aliphatic chain. This chain can either be saturated, or unsaturated. The nomenclature of fatty acids is such that although trivial names exist, all fatty acids can be described using the root name, i.e. that of the alkene or alkane, with the suffix -oic acid, for example octadecanoic acid.

In the accepted nomenclature of unsaturated fatty acids the position of the double bond is counted from the carboxyl acid end. Due to the complexity of naming fatty acids there is the conventional name of the fatty acid, i.e. tetradec-7-enoic acid, but also its omega classification. In fatty acids, omega, or ω, refers to the most distant carbon in the chain i.e. the one furthest from the carboxylic acid group. Therefore unsaturated fatty acids can be referred to by their ω designation with ω-3 referring to the carbon 3 positions away from the ω carbon and so on. The system of using a fatty acids ω name is useful when looking at the biosynthetic modification of such acids as the omega classification provides clarity on where the resulting double bond in an alkene will result. For example an ω-5 unsaturated fatty acid will form an alkene with the double bond in the 5 position, see Figure 6.1.

As previously discussed in Chapter 1 fatty acids are key molecules in the biosynthesis

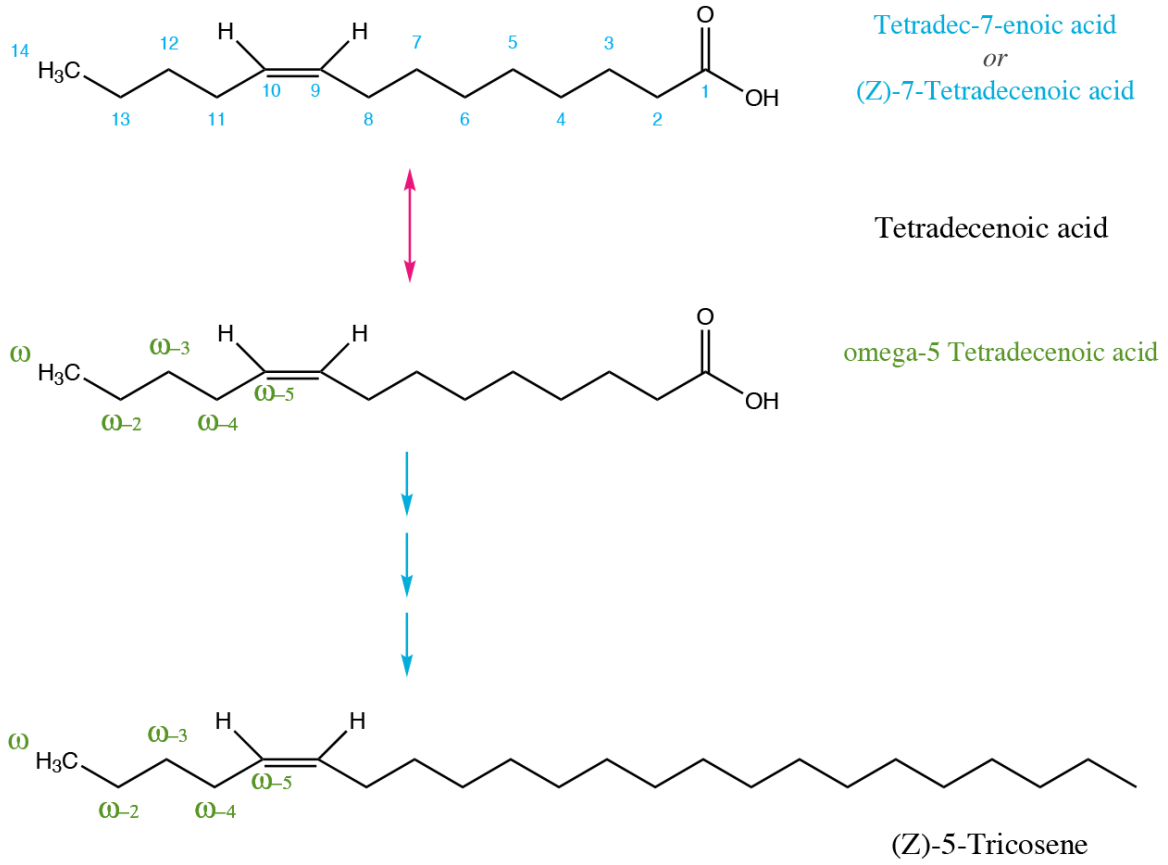


Figure 6.1: Schematic showing the labelling system of fatty acids, using the specific example of tetradecenoic acid. In this schematic, although the two fatty acids are the same molecule, they can have very different naming systems, with the different conventions shown using blue and green text. However it can be seen that the ω-x labelling system (green) indicates where the double bond position will be in the corresponding alkene. As indicated in this way a ω-5 acid will yield a (Z)-5-alkene. In the example shown (Z)-5-tricosene has been biosynthesised from the starting material ω-5 tetradecenoic acid.

of all types of cuticular hydrocarbons. Fatty acids can be obtained via two routes. Firstly fatty acids can be obtained through the consumption of dietary lipids, as part of fat containing molecules such as triacylglycerols [1]. The second route involves the *de novo* biosynthesis starting from just a single acetate molecule. During this process the length of the carbon chain is gradually extended in a cyclic reaction which involves adding an acetate molecule to the product of each cycle of the reaction. In this manner the chain length is extended by two carbons until the desired length is reached. See Figure 6.2 for a mechanism of this process.

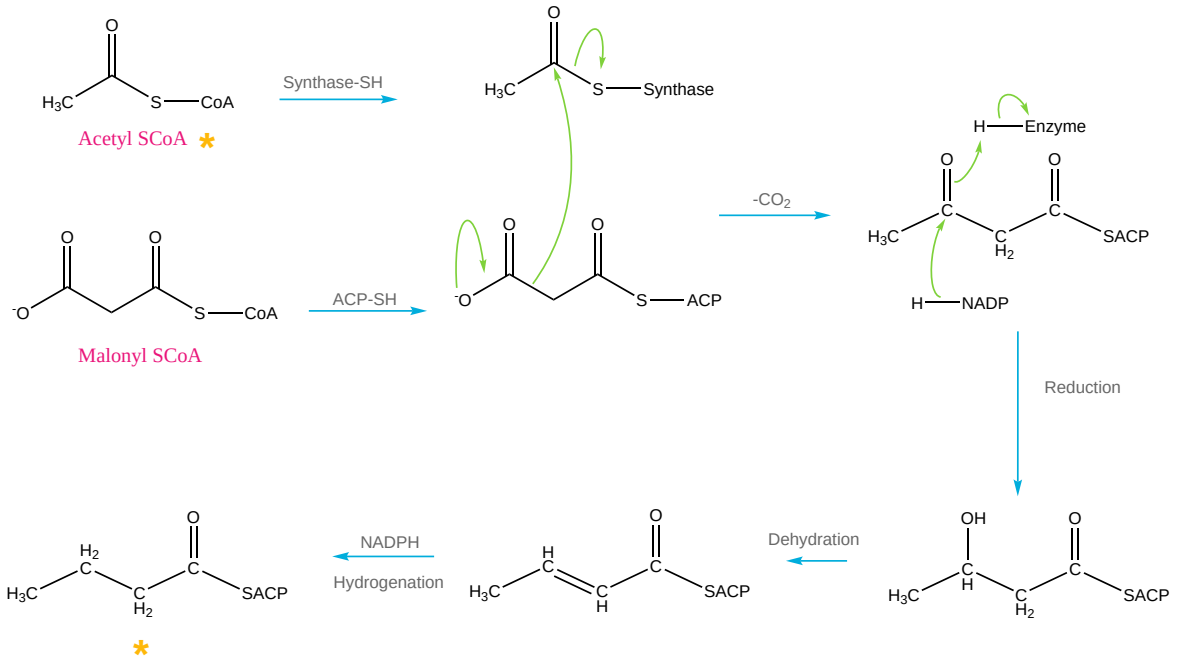


Figure 6.2: Reaction scheme showing the *de novo* formation of a fatty acid [2,3]. The product of this reaction indicated by a yellow asterisk can then act as starting material for the next reaction cycle, and thus the chain length is gradually increased.

The successful biosynthesis of long chain fatty acids is essential as they serve as a precursor to the biosynthesis of alkenes, alkanes and methyl-branched compounds. As Figure 6.3 outlines, in this synthesis a suitable fatty acid, in the form of a fatty acyl-CoA, either saturated or unsaturated, is extended in length. This extension continues until the chain length is one carbon greater than the chain length of the desired hydrocarbon. At this point a series of reactions occurs and the carbon containing the carbonyl group is removed to yield the desired hydrocarbon [2,3].

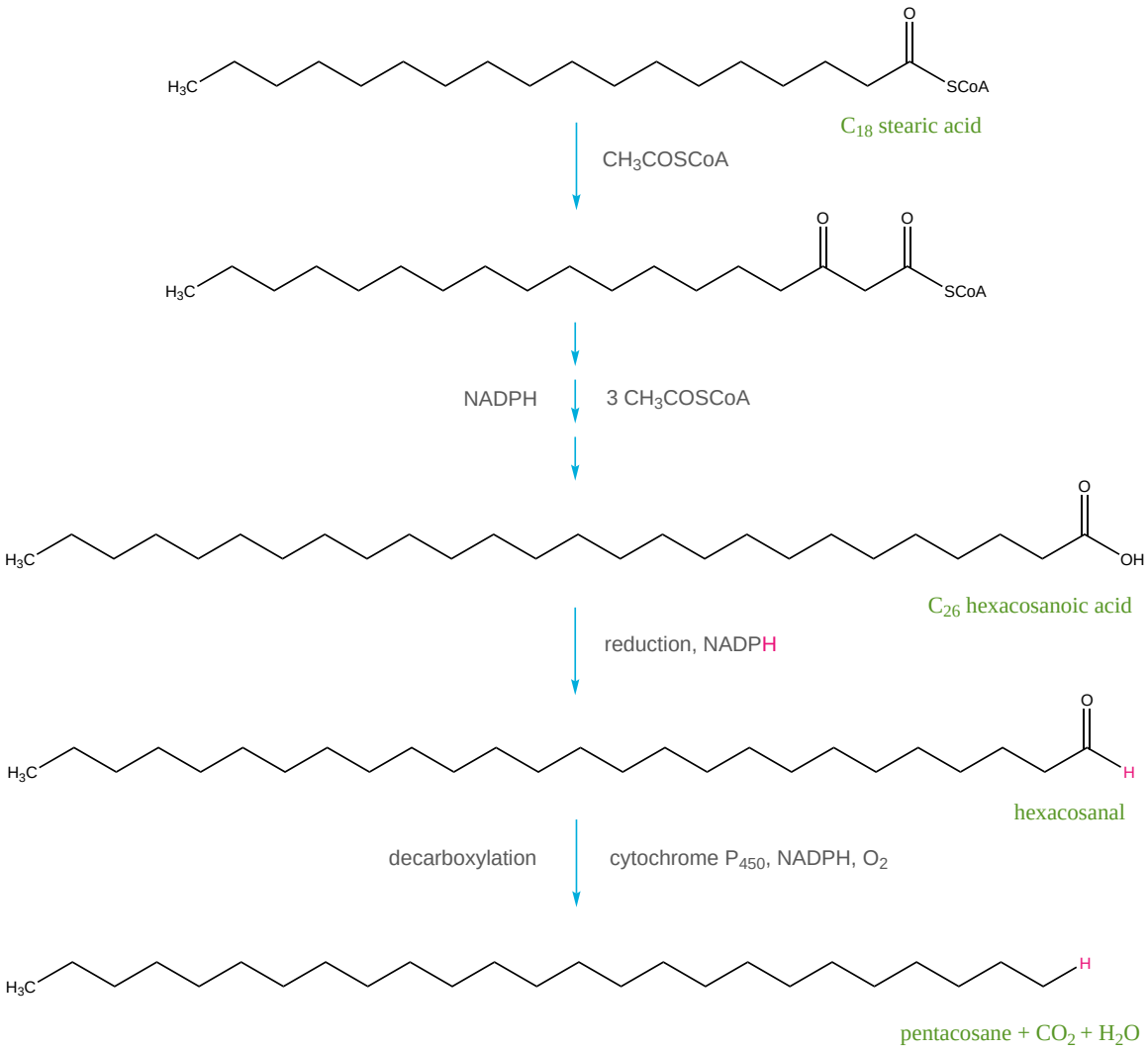


Figure 6.3: Outline biosynthesis of pentacosane via chain lengthening from stearic acid (C18). Adapted from [2].

Even though this is a widely understood biosynthetic route, there are still a few elements of this pathway which are less well known. The driving factor behind the formation of saturated and unsaturated cuticular hydrocarbons is still poorly understood. For example, the mechanism behind the formation of an alkene is not fully understood; it is not known whether in order to biosynthesise an alkene, a saturated fatty acid is used which is then dehydrogenated to form an unsaturated acid, or whether a suitable unsaturated fatty acid is used, such as oleic acid. Likewise, for the production of an alkane, it is not known whether an unsaturated fatty acid could be hydrogenated and subsequently extended. In research presented in Howard and Blomquist 2005 [4], the

authors suggest that the position of a double bond in an unsaturated hydrocarbon is determined by the fatty acyl desaturase which is used. Consequently many insects demonstrate alkenes with double bonds in the 9-position, due to the prevalence in nature of the Δ9-desaturase and the stearoyl-CoA precursor [4, 5]. This suggests that the formation of an alkene is driven by the formation of a saturated fatty acid which is then desaturated in the correct position to form an alkene of the correct positional isomer.

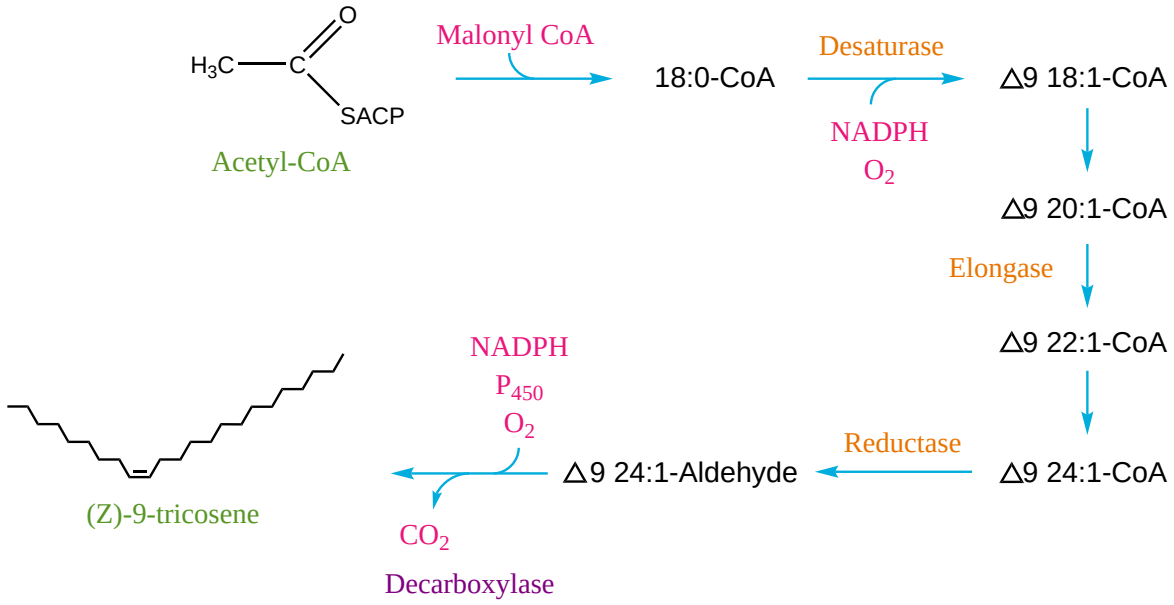


Figure 6.4: Reaction scheme showing the biosynthetic formation of a Z-9 alkene adapted from [4].

Whilst research suggests that this may be possible, previous unpublished work undertaken by the author of this thesis suggests that the biosynthesis of an unsaturated hydrocarbon can also occur using a suitable unsaturated fatty acid. This therefore shows that insect species can be resourceful in the precursors that they utilise and more research and experimentation is required in this area.

As previously discussed this project depends upon the availability of commercially

produced substrates with suitable stable isotope labels. Whilst a variety of suitable compounds exist they tend to be very expensive, and far beyond the budget for this project. There are many commercially synthesised fatty acids that exist, these can vary in terms of their chain length or double bond position, however the range of compounds available with suitable isotopic labels is small. Therefore it was decided that a selection of suitable fatty acids would need to be custom synthesised. This custom synthesis was performed by a third year undergraduate student.

Aims & Objectives

The primary aim of this chapter is to test whether small populations of wild *Myrmica sabuleti* incorporate a variety of synthesised fatty acids. These fatty acids vary in terms of both their chain length and label position however they are all unsaturated fatty acids, containing a single double bond. It is this double bond which acts as the deuterium labelling site. It is hoped that any trends that are related to the position of the label or the chain length can be discovered and in turn more information about the selection of fatty acids for biosynthesis can be discerned.

6.2 Experimental

In order to ensure a wide variety of unsaturated fatty acids were tested four different chain lengths were synthesised. These were; C14, C16, C18 and C20. The variety of fatty acids was further expanded by changing the positions of the double bond and deuterium labels. In total twelve different labelled fatty acids were synthesised, see Figure 6.5 for their structures.

The data obtained up to this point indicated that *Myrmica sabuleti* was a reliable species to work with. Individual extracts had yielded relatively simple, yet hydrocarbon rich profiles, containing a variety of compound types. Therefore it was decided that this experiment should focus on this species only. This experiment was done in three main blocks. The first block tested the substrate octadec-11-enoic acid-11,12-d_2, whilst the second focused on the substrates octadec-7-enoic acid-7,8-d_2 and hexadec-9-enoic acid-9,10-d_2. The final block involved the remaining nine fatty acids. For each individual substrate there were three repeat boxes, therefore in total for this experiment 36 plastic boxes measuring 17.0 x 11.5 x 4.5 cm were prepared by painting the sides with Fluon anti-stick coating (Blades Biological Ltd.) and the bottom with plaster of paris and a small amount of soil. All fatty acid experiments were run in the same manner using three repeat boxes per diet, with 25-30 *Myrmica sabuleti* ants per box.

As described later the substrate was added to the standard diet at a concentration of 3% w/w, rather than 2%. For these experiments there were no controls used, as this would have required a second, unlabelled batch of each fatty acid produced, and therefore twice the synthetic work. For this reason it was decided to modify the way in which the experiment was controlled. Instead, for the analysis of these results each experiment was compared against the values from 20 control *Myrmica sabuleti* extractions. These extractions were performed routinely when wild colonies were collected so that the chemical profiles could be used to differentiate between morphologically sim-

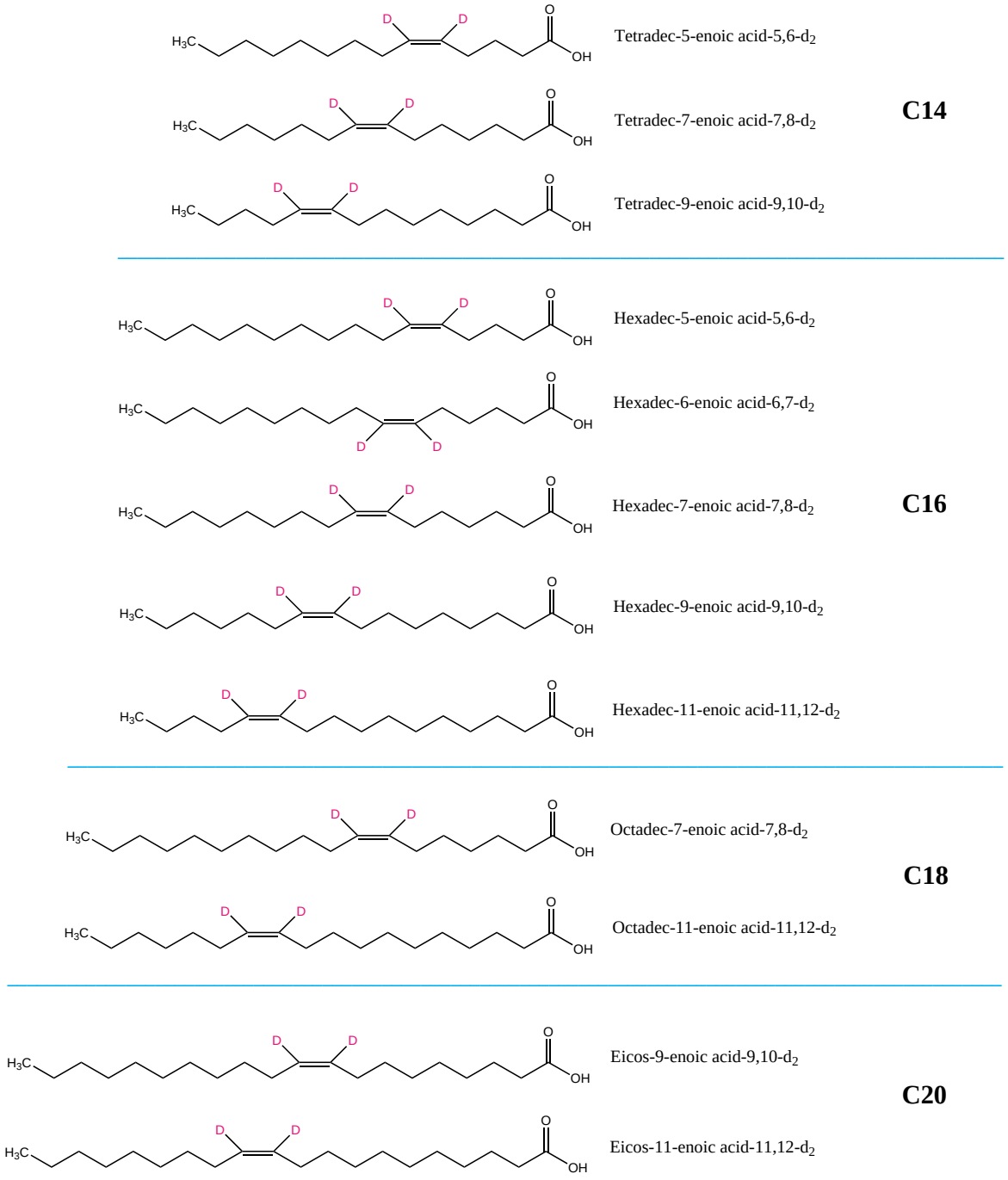


Figure 6.5: Structures of the fatty acids which were synthesised by an undergraduate student as part of a final year project. The fatty acids differ in the position of the double bond and subsequent deuterium labels.

ilar *Myrmica* species. By choosing these extractions as controls, a variety of colonies and individuals were used, this yielded a true representation of this species normal chemical profile.

In order to make the substrate diet a batch of standard diet was firstly prepared using the method provided in Section 3.1. Each substrate diet was made by adding approximately 100 μL (adjusted for density) of the required fatty acids to 3.3 g of standard diet to give a resulting concentration of 3% w/w. All diets were cut into small cubes (approximately 0.5 cm^3). One cube was placed on to a small piece of plastic inside each box and the food was replaced every other day. All diets were stored in the freezer and defrosted prior to use each time. All experiments were stopped after 30 days.

At this point all remaining ants from each of the boxes were extracted and analysed, according to the methods described in Sections 3.2.1 and 3.2.2. Initially six compounds were selected, again these were generally the most abundant but they also represented the three compound classes produced by this species; alkenes, alkanes and monomethyl-branched hydrocarbons. Once analysis of the raw data files had started it became apparent that a second pentacosene isomer was being biosynthesised in some cases. This was present in some of the chromatograms and ranged in intensity from a slight shoulder to a distinct peak. Whilst the presence of this secondary peak was by far the clearest within the results for octadec-11-enoic acid-11,12-d_2, it was felt that it would be appropriate to measure the ion abundances of the shoulder peak for all experimental substrates. See Figure 6.6 for a chromatogram which clearly shows this secondary pentacosene isomer.

This peak, which varied from a shoulder of the main pentacosene peak to a distinct separate peak, was designated pentacosene-2, as it was felt that this was a separate isomer to that which comprised the main peak, known as pentacosene-1. Therefore when analysing the chromatograms from this experiment seven compounds were analysed; those as described for *Myrmica sabuleti* in Section 4.3.3.1 and this secondary pentacosene isomer, known as pentacosene-2.

The measurement for this compound was taken at the highest point of the secondary

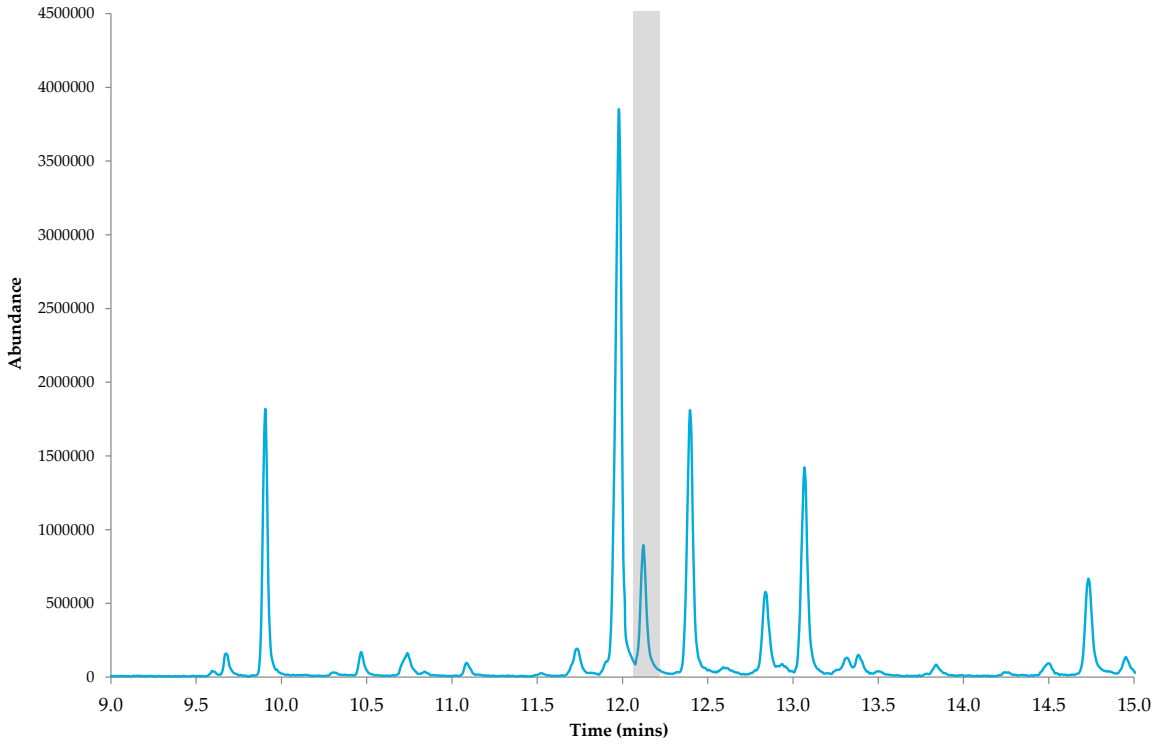


Figure 6.6: An example chromatogram showing the hydrocarbon profile of a C18 Z11 substrate fed *M. sabuleti* ant. The distinct secondary pentacosene peak, highlighted in grey, can clearly be seen and is known as pentacosene-2.

peak. This extra peak was only discernible within some of the chromatograms. Where there was no clear pentacosene-2 peak the measurement was made at the equivalent retention time relative to the peak for pentacosene. At this point all data was re-analysed and processed to ensure that this extra peak was included. Note that the individuals from each box were combined and treated as one data set as there was no discernible difference between groups. This also prevented the elimination of some groups from use because they didn't contain enough samples.

If incorporation was successful with these fatty acids then it was expected that the final mass of the hydrocarbon would increase by a maximum of two mass units. However it was also possible that during the course of biosynthetic modification to the fatty acid precursor, the deuterium labels would be themselves lost and replaced with hydrogen atoms. For this reason both M+1 and M+2 were analysed.

6.2.1 DMDS Analysis

In order to gain further information about the secondary isomer peak referenced above it was decided to run a DMDS reaction in order to potentially identify the structure of the extra isomer. In order to perform the DMDS reaction the 70 substrate samples obtained from the octadec-11-enoic acid-11,12-d_2 experiment (where the extra peak was clearest) were separated into 4 groups. Three of the groups contained twenty ants and these twenty were pooled together. The final group containing 10 ants were analysed individually. To each of the pooled extracts 200 μL of dimethyl disulfide and 100 μL of saturated iodine solution in diethyl ether was used. For the individual extracts 100 μL of dimethyl disulfide and 50 μL of saturated iodine solution in diethyl ether was used. Both methods were adapted from that by Carlson et al. [6]. The sample vials were heated overnight at 40 °C and saturated sodium thiosulfate solution was added to decolourise the iodine. The organic layer was extracted three times into a flat bottomed glass insert ready for analysis (50 μL of hexane added each time.) This was left to dry down until approximately 50 μL of solvent remained and the samples were injected. The same procedure was also used for the control samples, however this time the twenty control samples were divided into four pooled groups containing five ants each. The amounts of the reactants was as the individual substrate samples described above.

6.3 Results & Discussion

6.3.1 Tetradec-5-enoic acid-5,6-d_2

The following data refers to that obtained for tetradec-5-enoic acid-5,6-d_2. Note that for this experiment the number of samples for the substrate group was 21 and for all the fatty acid experiments the number of controls was 20.

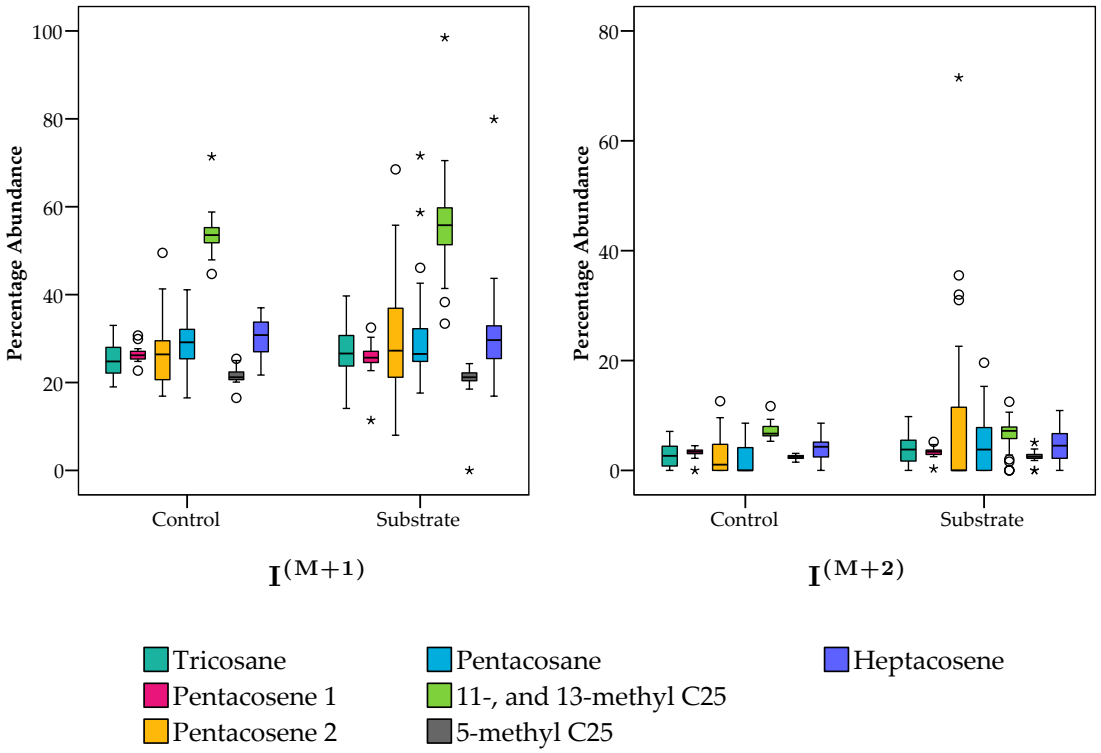


Figure 6.7: Boxplots showing the data for tetradec-5-enoic acid-5,6-d_2. The data for $I^{(M+1)}$ is on the left and $I^{(M+2)}$ on the right.

As can be seen from this data there does not appear to be any obvious difference between the two groups, with the median percentage abundance shown via the boxplots being similar between the groups. Table 6.1 shows the probability values as calculated using Mann-Whitney U-tests.

As the probability values indicate, there is statistical significance between the two groups for both studied alkane compounds but only at the $I^{(M+2)}$ level. It should also

Table 6.1: The probability values from the calculated Mann-Whitney U-tests for tetradec-5-enoic acid-5,6-d_2. Note that any p values less than 0.050, and therefore significant, are underlined for ease of reference.

Compound	p value	
	Tetradec-5-enoic acid-5,6-d_2	
	$I^{(M+1)}$	$I^{(M+2)}$
Tricosane	0.089	<u>0.043</u>
Pentacosene-1	0.104	0.346
Pentacosene-2	0.283	0.398
Pentacosane	0.444	<u>0.018</u>
11-, and 13-methyl C25	0.101	0.353
5-methyl C25	0.313	0.266
Heptacosene	0.440	0.231

be appreciated that the p value for tricosane at this level is 0.043, which although significant is only marginally so. This suggests that the ants may be able to use this labelled substrate as a precursor in the biosynthesis of alkanes. If this were the case it would lend support to the idea that these ants are able to manufacture a saturated hydrocarbon from an unsaturated fatty acid. However looking at the raw data there is no clear difference between groups. This suggests that any incorporation is very slight and hence only a minor pathway. The incorporation is only seen at the $I^{(M+2)}$ level, this is expected as hydrogenation of this labelled fatty acid to form the saturated equivalent would leave the two deuterium labels intact.

The chain length of this fatty acid is relatively short at just 14 carbons. Biologically it is less favourable for this compound to be used as a precursor at it requires more modification than a longer chain length, for example if this fatty acid was used as the starting material for the formation of pentacosane, then this process would first require that the acid is extended in length by 12 carbons, or six acetate molecules. The use of a longer fatty acid such as octadecenoic acid would only require the addition of four acetates and therefore would require less energy. This concept of energetic efficiency may therefore explain the low levels of incorporation seen. Use of this fatty acid as a

precursor may therefore depend on the availability of other acids and which are more energetically favourable.

However the tentative suggestion of its use as a precursor for just the alkanes is unexpected; if this compound is undergoing hydrogenation to form a saturated fatty acid then it is surprising that there is also no clear incorporation into the alkenes as this would require no extra modification. Although there is no difference according to the Mann-Whitney U-tests, there does appear to be some difference according to the raw data. As previously mentioned seven compounds were studied including two isomers for pentacosene as it was seen that this peak was producing a separate shoulder in some of the chromatograms. For the second of these pentacosene peaks, known as pentacosene-2, there were values for $I^{(M+2)}$ that were much higher than the comparable control values, see Appendices D.2-D.3 for the raw data. For example the range of the data for the ants fed with tetradec-5-enoic acid-5,6-d_2 for this peak at the $I^{(M+2)}$ level was 0.0 - 71.5%, the comparable range for the control was 0.0 - 12.6%. When looking at the raw data it is useful to consider the mean percentage abundances for the two groups. These were 8.2% for the substrate and 3.1% for the control respectively. Within the substrate group there were six samples with a value greater than 20%, whereas there were no samples in the control group satisfying this criteria. In addition there were no values greater than 20% shown for any other compounds at this level, indicating that this change was specific to this isomer of pentacosene. This shows that there were some individual ants within the substrate group that appear to show clear incorporation for this compound at this level, but as this is not a change seen across the population, the statistical analysis does not reflect this. There are a number of factors which could be attributing to this selective incorporation amongst individuals and these will be discussed in greater detail at the end of this chapter.

6.3.2 Tetradec-7-enoic acid-7,8-d_2

The following data refers to the data obtained for tetradec-7-enoic acid-7,8-d_2. Note that for this experiment n=53.

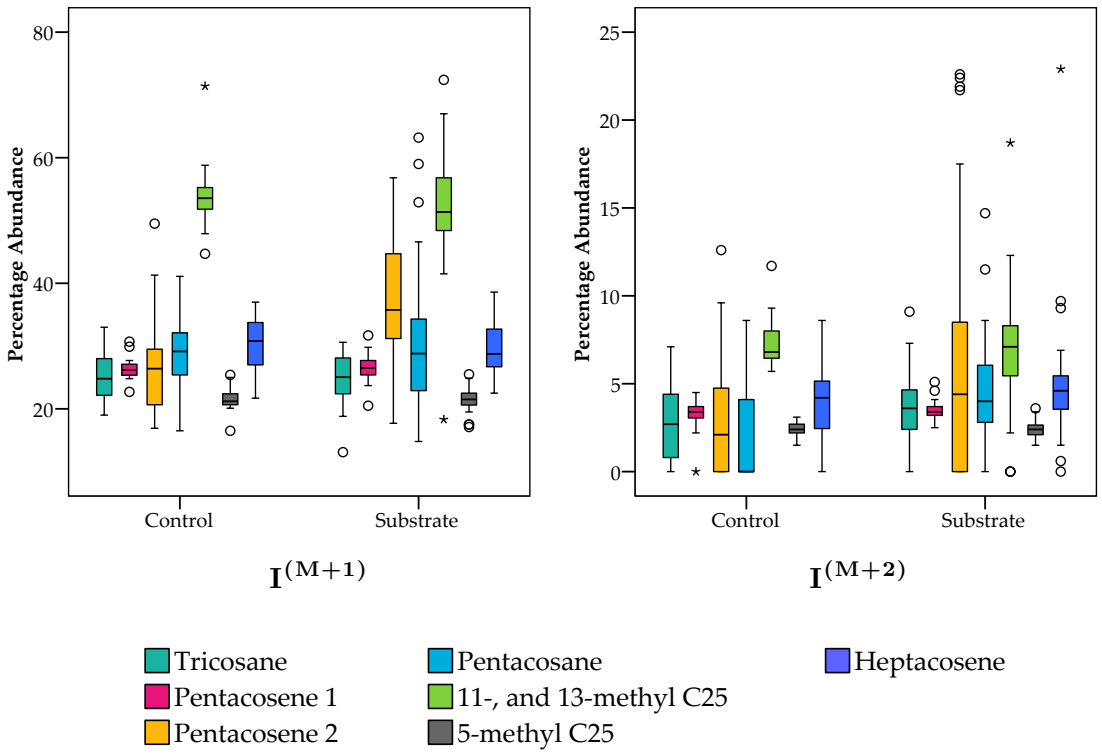


Figure 6.8: Boxplots showing the data for tetradec-7-enoic acid-7,8-d_2. The data for $I^{(M+1)}$ is on the left and $I^{(M+2)}$ on the right.

As Figure 6.8 shows there appears to very little difference between the two sets of data. However when looking at the data for $I^{(M+1)}$ it can be seen that the median percentage abundance for the substrate data and the pentacosene-2 compound appears to be much greater than the equivalent control value. Pentacosene-2 refers to an additional pentacosene isomer which was present in some of the data files. Analysis of the raw data shows that the mean percentage abundances for this compound are very different within the two data sets with calculated values of 37.5% and 27.0% for the substrate and control respectively. See Appendices D.4-D.5 for the raw data.

For the $I^{(M+2)}$ data there appears to be a difference between the percentage abundance

values for pentacosane, with the substrate data showing a greater median than the control. This difference is reflected in the raw data with mean percentage values of 6.3% and 3.1% for substrate and control respectively. Further statistically significant differences between groups can be identified between groups using Mann-Whitney U-tests, see Table 6.2.

Table 6.2: The probability values from the calculated Mann-Whitney U-tests for tetradec-7-enoic acid-7,8-d_2. Note that any p values less than 0.050, and therefore significant, are underlined for ease of reference.

Compound	p value	
	Tetradec-7-enoic acid-7,8-d_2	
	$I^{(M+1)}$	$I^{(M+2)}$
Tricosane	0.487	<u>0.027</u>
Pentacosene-1	0.326	0.271
Pentacosene-2	<u>0.000</u>	0.510
Pentacosane	0.402	<u>0.005</u>
11-, and 13-methyl C25	0.136	0.309
5-methyl C25	0.457	0.350
Heptacosene	0.318	0.088

Table 6.2 shows that there are some statistical differences between the two groups. The results from the Mann-Whitney U-tests show that there is a difference in the pentacosene isomer peak for $I^{(M+1)}$, with a calculated p value of 0.000. For the $I^{(M+2)}$ data there are differences indicated in the tricosane and pentacosane incorporation.

As with all of these fatty acid substrates incorporation of one fatty acid molecule should produce a resulting hydrocarbon which is two mass units heavier than its unlabelled equivalent. However this experiment utilised deuterium labels and whilst carbon-deuterium bonds are not particularly labile exchange with a hydrogen atom cannot be ruled out. The minor possibility of hydrogen exchange may explain why mass changes of just one unit are sometimes observed, such as that for pentacosene-2. Another factor that might contribute to the addition of a single deuterium atom is the process of the

deuteration. This is achieved via the use of deuterium gas, however the purity of this reagent was not 100% and, as such, some hydrogen atoms would have been added as well as deuterium atoms. However it should be mentioned that this would only account for a very small amount of the $I^{(M+1)}$ observed here; MS data for the synthesised fatty acids indicated sucessful levels of deuteration.

The apparent incorporation of deuterium labels into the two alkane compounds at the $I^{(M+2)}$ level is an interesting observation. The statistical difference between the control and the substrate for these compounds at this level indicates that a greater amount of alkane molecules that were two mass units heavier were detected in the substrate samples compared to the control. This suggests that this difference is down to the usage of the labelled fatty acids when biosynthesising alkane compounds. However if this is the cause then it indicates that ants are able to use an unsaturated fatty acid in the biosynthesis of a saturated hydrocarbon. This result shows the same trend as that seen for the other related substrate, tetradec-5-enoic acid-5,6-d_2, and therefore corrobates the results observed previously.

6.3.3 Tetradec-9-enoic acid-9,10-d_2

The following data refers to the data obtained for tetradec-9-enoic acid-9,10-d_2. Note that for this experiment the number of substrate samples was 25. See Figure 6.9.

Looking at the data for this experiment, at the $I^{(M+1)}$ level there does not appear to be much difference between the two groups. However at the $I^{(M+2)}$ level there appears to be greater discernible difference between the two; the median percentage abundance for pentacosane in the substrate group is much greater than the comparable value for the control. This is also reflected in the raw data, with mean values of 4.3% and 2.2% for the substrate and control respectively. See Appendix D.6 for this data.

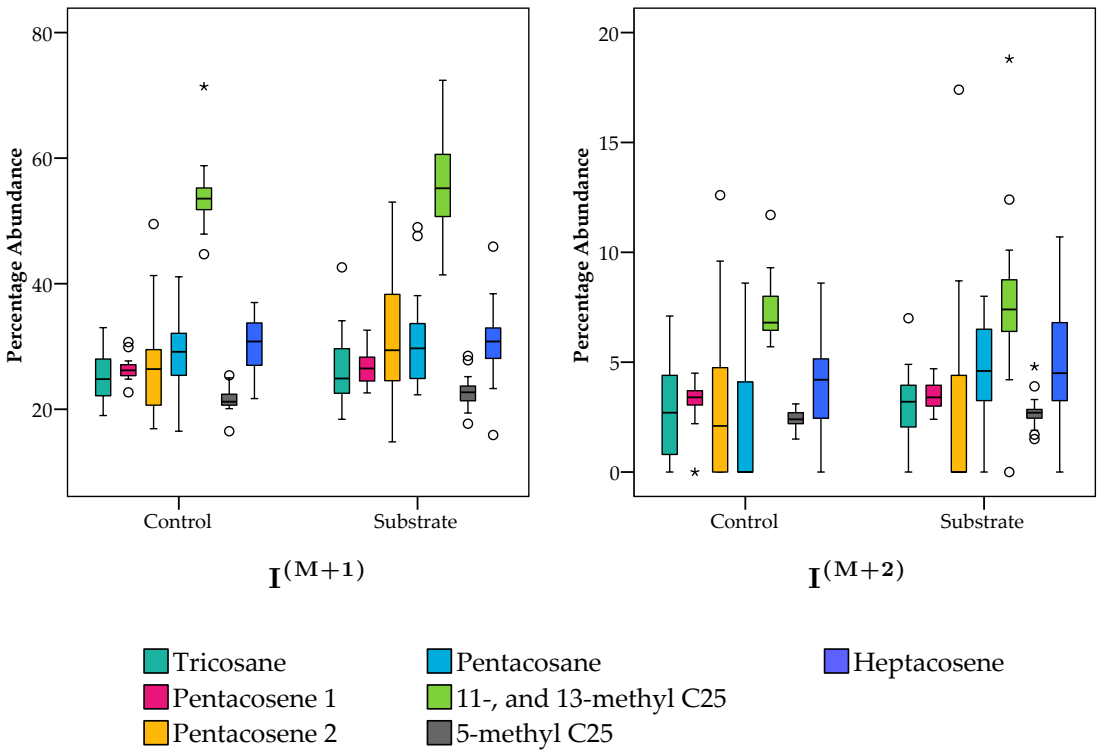


Figure 6.9: Boxplots showing the data for tetradec-9-enoic acid-9,10-d_2. The data for $I^{(M+1)}$ is on the left and $I^{(M+2)}$ on the right.

The results of the calculated Mann-Whitney U-tests provide a much clearer indication of any differences in the percentage abundance values between the control and the substrate groups. Table 6.2 shows the probability values as calculated using Mann-Whitney U-tests for all studied compounds at both measured isotope levels.

As the results of these calculations show there is some statistical difference indicated between the two groups. There are two compounds which show incorporation at the $I^{(M+1)}$ level, these are the secondary isomer of pentacosene and 5-methylpentacosane (5-methyl C25). The incorporation indicated for 5-methylpentacosane is very unusual as the normal route of biosynthesis for this compound would be the *de novo* synthesis of a branched fatty acid using propionate and acetate (see Section 1.2.2.4). However there is a difference in the groups with relation to this compound, which suggests that the labelled substrate is somehow incorporated. It should be noted that the value for p is 0.031, which is not much lower than the 95% confidence interval of 0.050. There is no theory as to why this branched hydrocarbon would show incorporation of this

Table 6.3: The probability values from the calculated Mann-Whitney U-tests for tetradec-9-enoic acid-9,10-d_2. Note that any p values less than 0.050, and therefore significant, are underlined for ease of reference.

Compound	p value	
	Tetradec-9-enoic acid-9,10-d_2	
	$I^{(M+1)}$	$I^{(M+2)}$
Tricosane	0.254	0.261
Pentacosene-1	0.365	0.301
Pentacosene-2	<u>0.046</u>	0.189
Pentacosane	0.457	<u>0.009</u>
11-, and 13-methyl C25	0.118	0.277
5-methyl C25	<u>0.031</u>	0.072
Heptacosene	0.464	0.220

labelled substrate as it does not fit with any known biosynthesis model.

Statistical significance was also seen for the secondary isomer of pentacosene, but only at the $I^{(M+1)}$ level. Again this value was only slightly lower than the critical value of p, which indicates that there was only a slight difference between the groups. Appendix D.6 shows the raw data. Looking at this data it can be seen that the range of the data is much greater for the substrate group, this can be also seen from the relevant boxplot in Figure 6.9. The percentage abundance values for the 25 samples in the substrate group for this compound, at this level, varied from 14.8% to 53.0%. In comparison the same range for the control data was 16.9% to 49.5%. Whilst this range is very similar between the two groups they differ in the frequency of the higher values, for the substrate group 20% of the samples had a measured percentage abundance greater than 40% at the $I^{(M+1)}$ level. This is compared to 10% for the control group. The mean values reflect this difference with 31.9% and 27.0% for substrate and control respectively. For reference, according to Equation 3.2 the normal percentage abundance value for $I^{(M+1)}$ level would be 27.5%. Given these measurements it can be suggested that there is incorporation of this labelled fatty acid into this isomer of pentacosene, however this only appears to be a mild effect and is only demonstrated

by some individuals within the group. Previously with tetradecenoic acid substrates, incorporation was seen for the alkanes tricosane and pentacosane at the $I^{(M+2)}$ level, this trend is partially repeated in this set of results, with statistical significance seen only for pentacosane with this substrate.

Tetradecenoic acid overview

Looking at the results for all the tetradecenoic acid substrates as a whole reveals some trends. It seems that for this substrate if incorporation is present then it is mainly seen for the alkane compounds. This suggests that here the ants are using unsaturated fatty acids and hydrogenating them to form saturated compounds. This goes against the accepted model of the biosynthesis of alkanes, which is that they are formed from saturated fatty acids. Whilst this is what the data suggests it should be clarified that if this is what is happening this is only a minor biosynthetic route, as the raw data does not support this being the major route of biosynthesis. If this were the case and that alkanes were routinely sythesised using unsaturated precursors then it would be expected that incorporation would be much more widespread with much greater mean percentage incorporation values.

The elongation of a fatty acid containing fourteen carbon atoms is not as biologically favourable as the elongation of a fatty acid containing, for example, 18 carbon atoms, as this will require less elongation and thus less energy. However this seemingly less favourable route of synthesis may simply be down to the amounts of precursors which are available. Under normal conditions these ants would be consuming a mixed diet containing a wide variety of fatty acids, however instead these ants have a fairly limited diet of which 3% by mass is a fatty acid. Therefore the apparent use of this compound may be down to its availability, and because of its abundancy within the ants' diet it is the utilised precursor, despite the fact it is not as biologically efficient. This may suggest that availability is the driving factor in precursor usage rather than efficiency.

Although there are no clear trends it does seem like these fatty acids are used in the biosynthesis of alkanes, which is not what was expected. It was assumed that these unsaturated compounds would be used in the biosynthesis of unsaturated cuticular hydrocarbons. However this is not always the case, although incorporation was seen for tetradec-7-enoic acid-7,8-d_2 and tetradec-9-enoic acid-9,10-d_2 in the secondary pentacosene isomer at the $I^{(M+1)}$ level. Incorporation was also seen for tetradec-5-enoic acid-5,6-d at the $I^{(M+2)}$ level.

When naming fatty acids the position of the double bond is counted from the carboxyl acid end. However as previously mentioned the nomenclature of an alkene is slightly different in that the count is made from whichever end gives the smaller number. When these fatty acids become alkenes, elongation occurs by adding a carbon to the carboxyl group end of the chain, therefore the relative position of the double bond in the fatty acid remains unchanged. However when decarboxylation occurs the fatty acid group is removed and the alkene is formed. According to the naming convention of alkenes the denoted position of the double bond may change, due to counting from the other end of the chain. See Figure 6.10. This apparent change can lead to confusion; in the example provided in Figure 6.10 it may seem that the double bond position has changed as tetradec-7-enoic acid forms (Z)-5-tricosene. Therefore it is important to refer to these fatty acids both by their conventional name and their ω designation. This is a useful additional way of classifying these fatty acids as a ω-9 will form an alkene with the double bond in the 9 position and so on. In this way the fatty acids tetradec-7-enoic acid-7,8-d_2 and tetradec-9-enoic acid-9,10-d_2 are ω-5 and ω-7 respectively. Referring to these fatty acids by their omega classification therefore provides clarity on where the resulting double bond in an alkene will result.

On this basis the acids tetradec-7-enoic acid-7,8-d_2 and tetradec-9-enoic acid-9,10-d_2 are classed as ω-5 and ω-7 respectively and thus would form Z5 and Z7 alkenes respectively. The species used in this experiment was *Myrmica sabuleti*, the double bond

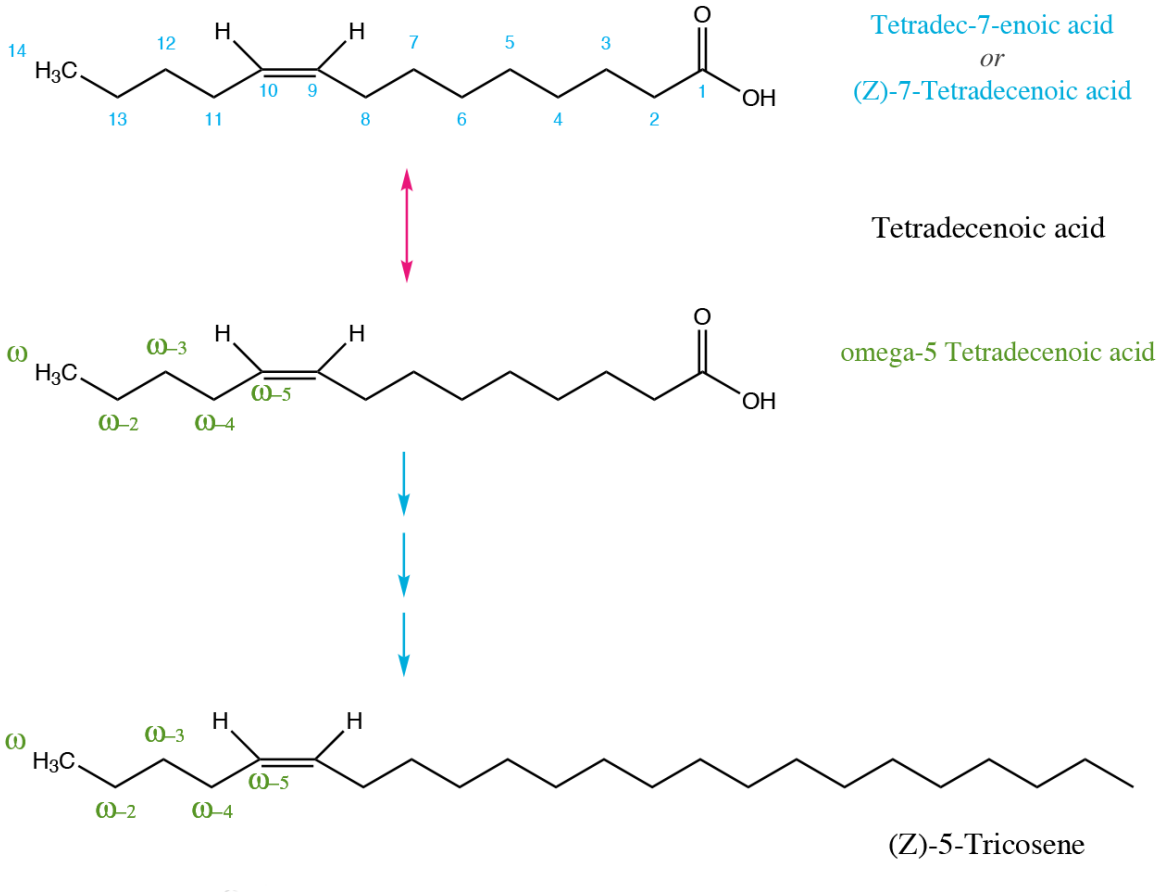


Figure 6.10: Schematic showing the labelling system of fatty acids, although the two fatty acids are the same molecule they can have very different naming systems. However it can be seen that the ω-x labelling system indicates where the double bond position will be in the corresponding alkene. As shown in this way a ω-5 acid will yield a (Z)-5-alkene.

positions of which, in pentacosene, have already been analysed and found to be Z9, Z11 and Z12, see Table 3.1. Therefore on this basis, neither tetradec-7-enoic acid-7,8-d_2 and tetradec-9-enoic acid-9,10-d_2 should be suitable precursors because their usage would result in a Z5 or Z7 pentacosene, a double bond position which is not found in *Myrmica sabuleti*. This however may begin to explain the incorporation seen for alkanes, and will be further discussed later in this chapter.

6.3.4 Hexadec-5-enoic acid-5,6-d_2

The following data refers to the data obtained for hexadec-5-enoic acid-5,6-d_2. Note that for this experiment the number of samples for the substrate group was 30.

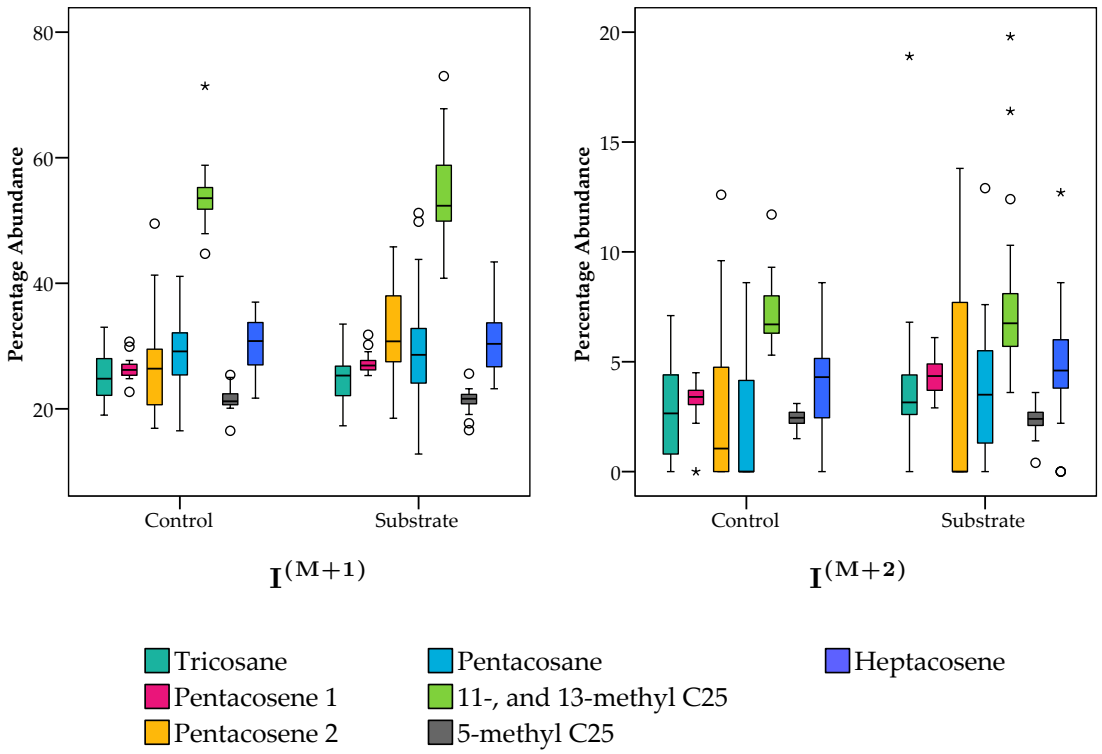


Figure 6.11: Boxplots showing the data for hexadec-5-enoic acid-5,6-d_2. The data for $I^{(M+1)}$ is on the left and $I^{(M+2)}$ on the right.

The boxplots show that the data between the two groups is fairly similar, although there do appear to be some differences between the median percentage abundance values. However this is not always indicative of any statistical significance. In order to identify any differences between the groups Mann-Whitney U-tests were performed on the data.

Table 6.1 shows the probability values as calculated using Mann-Whitney U-tests. As these values show there is a statistical difference for both pentacosene isomers but only at the $I^{(M+1)}$ level. Additionally at the $I^{(M+2)}$ level there are two compounds showing statistical significance, these are the primary pentacosene isomer and the alkane pentacosane.

Table 6.4: The probability values from the calculated Mann-Whitney U-tests for hexadec-5-enoic acid-5,6-d_2. Note that any p values less than 0.050, and therefore significant, are underlined for ease of reference.

Compound	p value	
	Hexadec-5-enoic acid-5,6-d_2	
	$I^{(M+1)}$	$I^{(M+2)}$
Tricosane	0.401	0.075
Pentacosene-1	<u>0.027</u>	<u>0.000</u>
Pentacosene-2	<u>0.003</u>	0.395
Pentacosane	0.455	<u>0.041</u>
11-, and 13-methyl C25	0.397	0.356
5-methyl C25	0.382	0.349
Heptacosene	0.490	0.071

Looking at the data for $I^{(M+1)}$ initially, from these values the smaller p value is associated with the secondary pentacosene isomer. The difference between groups is reflected in the mean percentage abundance values from the raw data with values of 32.7% for the substrate and 27.0% for the control. According to Equation 3.2 a typical control value for this compound would be 27.5%, so this control value is reflective of this. Looking at the 25 substrate samples that make up this data set it can be seen that there are some values for the secondary isomer of pentacosene that are similar to this value, however there are several values which are much higher than this. See Appendices D.7-D.8 for the raw data. This suggests that this data is subject to individuals showing a particular result, as opposed to an entire group showing a change. In this case it seem that some of the tested individuals incorporate the labelled fatty acid into this alkene isomer and some do not. There are a number of factors which could affect this, and these will be discussed in greater detail at the end of the chapter.

At the $I^{(M+2)}$ level there are two compounds showing significance, the primary pentacosene isomer and also pentacosane. Although the p value for pentacosene and $I^{(M+1)}$ is only 0.027, the value at the $I^{(M+2)}$ level is 0.000. If there is true incorporation of the labelled fatty acid into this compound then it might well be expected that the p

value for $I^{(M+1)}$ would be greater than the value for $I^{(M+2)}$ i.e. $I^{(M+1)}$ would be less statistically significant. This is because when a molecule of the labelled fatty acid is incorporated the resulting alkene is two mass units heavier and this is detectable in the $I^{(M+2)}$ measurement. In order for the level of $I^{(M+1)}$ to increase a mass increase of one unit must occur. This could be from the incorporation of a partially labelled fatty acid, i.e. only one label present, or the incorporation of a doubly labelled fatty acid which subsequently loses one label via hydrogen exchange during the biosynthetic process. However these processes will be much less frequent and consequently the p values for the $I^{(M+2)}$ measurement should be much lower. It is also worth considering that the $I^{(M+2)}$ measurement is also more inherently sensitive as normal control values are very low, therefore even slight increases to this value would be easily identified. Additionally looking at the raw data suggests that there are no individuals with very high percentage abundances, more a general trend across the entire group. Therefore the p values at both measured levels can therefore be seen as an indication of the results reliability.

The results therefore reliably indicate that this labelled fatty acid may be being incorporated into the primary biosynthesised alkene, pentacosene-1. The known isomers of *Myrmica sabuleti* for the compound pentacosene are Z9, Z11, and Z12, see Table 3.1. The labelled fatty acid hexadec-5-enoic acid-5,6-d_2 is a ω-11 fatty acid and would therefore produce a Z11 isomer, so it is possible that this is what is being observed here; the provided fatty acid can be easily biosynthesised into Z11 pentacosene. However this is present in small amounts, and therefore would not represent a significant biosynthetic pathway for this species. It is also worth mentioning that within this species a Z11 isomer was only identified for pentacosene and therefore, following the previous theory, incorporation would not be expected for heptacosene as this would require a ω-13 fatty acid to biosynthesise the target Z13 heptacosene isomer.

Significance is also seen for pentacosane. It is again possible that the unsaturated

fatty acid is being hydrogenated to form a saturated compound, a process, which as previously discussed, may be driven by the high abundance of the fatty acid. It is also interesting to note that so far all the tested fatty acid substrates have shown a significant p value for pentacosane at the $I^{(M+2)}$ level, a trend which cannot be ignored.

6.3.5 Hexadec-6-enoic acid-6,7-d_2

This labelled substrate (sapienic acid) is not widely found in nature, apart from as a component of *Thunbergia alata* seed oil [7]. It is a ω-10 acid and would therefore yield a Z10 positional isomer, a position which is not found in *M. sabuleti*. For this substrate the number of samples was 35. For the box plots of this data see Figure 6.12. The boxplots show that the range of the data is similar between groups for both plots however there is a difference in the median percentage abundance for the secondary isomer of pentacosene, denoted in the graph by pentacosene-2 (yellow box).

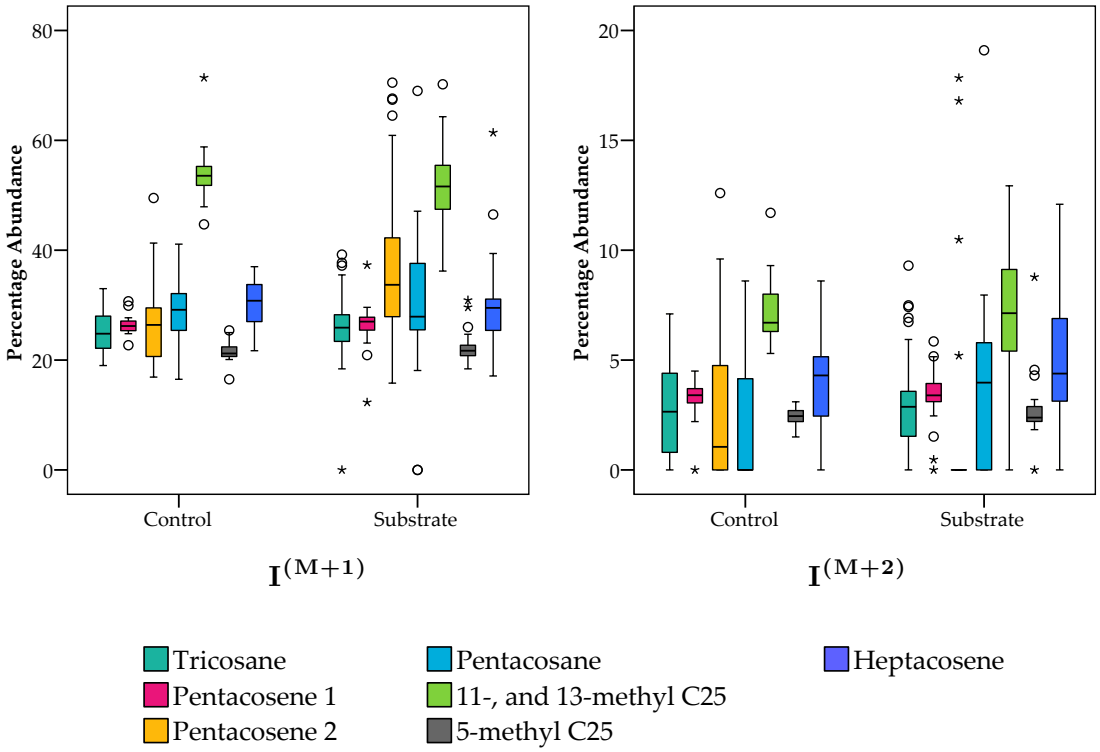


Figure 6.12: Boxplots showing the data for hexadec-6-enoic acid-6,7-d_2. The data for $I^{(M+1)}$ is on the left and $I^{(M+2)}$ on the right.

In order to determine any statistically significant differences between the two groups Mann-Whitney U-tests were performed on the data. For a table of the results please see Figure 6.5.

Table 6.5: The probability values from the calculated Mann-Whitney U-tests for hexadec-6-enoic acid-6,7-d_2. Note that any p values less than 0.050, and therefore significant, are underlined for ease of reference. Any p values less than 0.050 which are italicised may be disregarded and are due to the median rank of the control being greater than that of the substrate group.

Compound	p value	
	Hexadec-6-enoic acid-6,7-d_2	
	$I^{(M+1)}$	$I^{(M+2)}$
Tricosane	0.242	0.382
Pentacosene-1	0.191	0.312
Pentacosene-2	<u>0.001</u>	*0.003*
Pentacosane	0.373	0.052
11-, and 13-methyl C25	0.099	0.467
5-methyl C25	0.315	0.426
Heptacosene	0.251	0.261

The results of the U-tests indicate that there was only one compound which was statistically significant within this experiment and this was the secondary pentacosene isomer at the $I^{(M+1)}$ level, with a p value of 0.001. However the data also indicates that there was statistical significance at the $I^{(M+2)}$ level, however this was in the wrong direction. All quoted p values are one-tailed, as the only important significance is whether the substrate percentage abundances are greater. This data is indicating that for this compound at this level the control values were higher. This can be seen by looking at the raw data, see Appendices D.9-D.10. The average percentage abundance for this compound for the control was 3.1%, compared to the substrate, which was 1.4%. This is likely due to the overall lower quality of the substrate data compared to the control; the lower mean value was caused by a data set with many more individuals with percentage abundance values of 0.0%. A further check of the raw data shows that

the substrate data has many ion count values for pentacosene which are very low, and hence highly unreliable.

As previously discussed there are two main factors which can cause low ion counts. Firstly it could be down to the instrument; a dirty ion source for example is known to cause poor sensitivity [8]. However this is less likely to be the cause of the issue as all the samples were run together, therefore this would be an issue stretching across individual experiments. More likely is that the low ions counts are due to the individual ants themselves and the amounts of hydrocarbons present on the cuticle. There are several studies investigating the relationship between environmental factors and hydrocarbon production in both ant and non-ant species [9–11]. A study by Wagner et al. [10] investigated the difference in CHC composition between forager and non-forager *Pogonomyrmex barbatus* ants. They related differences in the cuticular hydrocarbons of forager and non-forager ants to differences in temperature and humidity and the effects that these variables have on CHC profiles. Whilst care is taken to ensure that each box is the same environment, there are still many variables such as humidity which cannot be tightly controlled and therefore this could have caused low amounts of CHCs to be present on the cuticle.

Given that the results indicate inaccuracy it would be prudent to assume that the result for the significance of pentacosene-2 at the $I^{(M+1)}$ level is also inaccurate and should be disregarded. Although the ion counts are low, the raw data sheet indicates that this data is not inaccurate; the difference in averages is large with an average percentage abundance value of 37.9% for the substrate data, compared to 27.0% for the control. The range of the data for the substrate is also much greater; and within the substrate group 31% of the percentage abundance values are greater than 40%, compared to just 10% of the control data. Given this, it seems that this is a genuine result, it can therefore be suggested that the ants were able to incorporate the labelled substrate hexadec-6-enoic acid-6,7-d_2 into a secondary isomer of pentacosene. Hexadec-6-enoic

acid-6,7-d_2 is an ω-10 acid and would therefore yield a Z10 positional isomer, a position which is not found in *M. sabuleti*, this may explain why incorporation is not seen for the primary isomer of pentacosene, and may suggest that the secondary peak is a different isomeric position to that usually found in the species.

6.3.6 Hexadec-7-enoic acid-7,8-d_2

Hexadec-7-enoic acid, also known as hypogeic acid, is uncommon in nature. The following data refers to the data obtained for hexadec-7-enoic acid-7,8-d_2. Note that for this experiment the number of samples for the substrate group was 38.

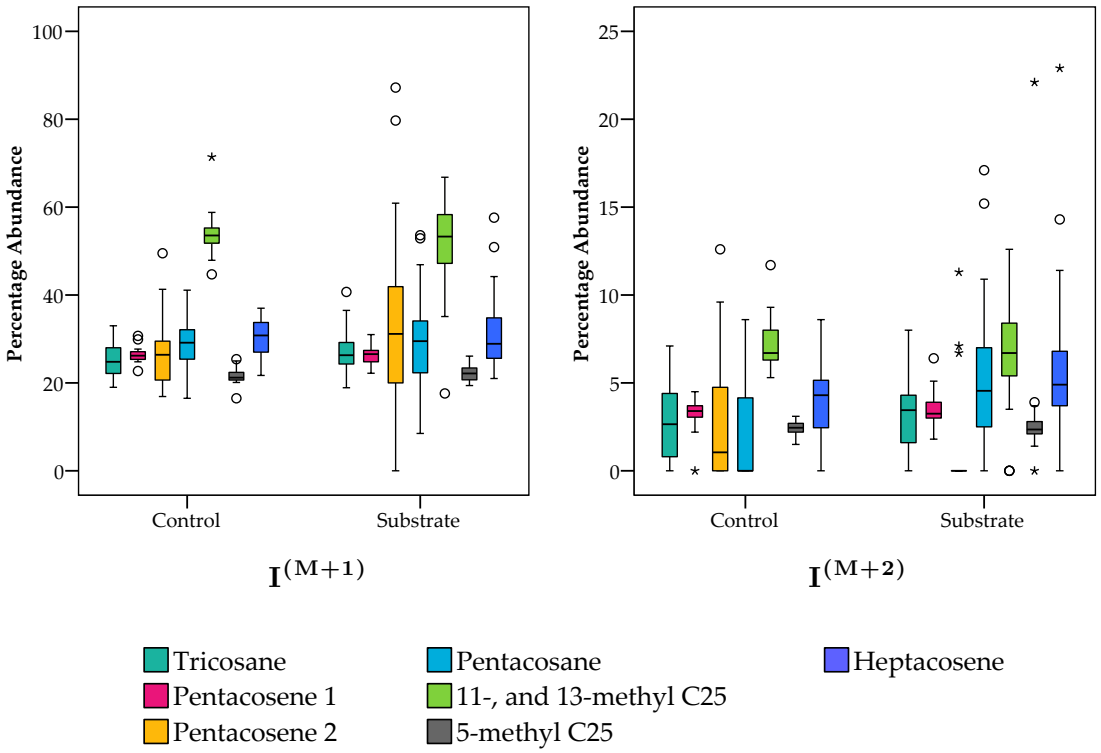


Figure 6.13: Boxplots showing the data for hexadec-7-enoic acid-7,8-d_2. The data for $I^{(M+1)}$ is on the left and $I^{(M+2)}$ on the right.

The data represented in these boxplots shows that there does not appear to be any clear difference between the groups. Although the median for the secondary pentacosene peak is higher in the substrate group than the control group for $I^{(M+1)}$, the

range of the data is much greater for the substrate. There does however appear to be a clear difference in the pentacosane data for the $I^{(M+2)}$ data. Mann-Whitney U-tests were performed on this data to determine any significance between the groups.

Table 6.6: The probability values from the calculated Mann-Whitney U-tests for hexadec-7-enoic acid-7,8-d_2. Note that any p values less than 0.050, and therefore significant, are underlined for ease of reference. Any p values less than 0.050 which are italicised may be disregarded and are due to the median rank of the control being greater than that of the substrate group.

Compound	p value	
	Hexadec-7-enoic acid-7,8-d_2	
	$I^{(M+1)}$	$I^{(M+2)}$
Tricosane	0.089	0.199
Pentacosene-1	0.431	0.386
Pentacosene-2	0.097	*0.000*
Pentacosane	0.453	<u>0.002</u>
11-, and 13-methyl C25	0.329	0.295
5-methyl C25	0.152	0.386
Heptacosene	0.469	0.063

The results of the U-tests indicate that there is a significant difference in the percentage abundance values for pentacosane at the $I^{(M+2)}$ level. This is in keeping with the trend that has been observed so far which is that analysis at the $I^{(M+2)}$ level tends to indicate incorporation for pentacosane. The reasons for this are not yet fully understood but will be discussed in greater depth at the end of this chapter.

Again however it can be seen that there is a significant difference between the control and substrate data for pentacosene-2, but in the wrong direction. Looking at the raw data sheet reveals that this result is again due to many percentage abundance values of 0.0% for the substrate. A value of 0.0% is recorded if there is no ion count present for that measurement. Again this is likely to be because of low sensitivity or low amounts of CHCs present.

It is worth considering that as this is an ω-9 acid it would potentially be used by *Myrmica sabuleti* to biosynthesise a Z9 alkene. However as previously discussed, within this species Z9 is only a minor positional isomer, see Table 3.1. Therefore it is possible that supplies of this substrate were not required during the course of the experiment for biosynthesis. As previously mentioned, for this experiment wild ants were collected specifically for this purpose, therefore it can be assumed that they would have had access to a full and varied diet. This may mean that natural stores of biologically important molecules would still be high due to these being recently collected wild ants. The lack of incorporation observed however could also be because a route for the use of this fatty acid to biosynthesise unsaturated alkenes does not exist, because this fatty acid is so uncommon in nature.

6.3.7 Hexadec-9-enoic acid-9,10-d_2

Within nature hexadec-9-enoic acid is fairly prevalent and is known by the common name palmitoleic acid. Palmitoleic acid is widely found in nature in animals, plants, micro-organisms and seed oils [12, 13]. The diet of *Myrmica sabuleti* is known to contain small insects and seeds and so palmitoleic acid likely makes up an important part of their nutrient intake [14, 15]. The following data refers to that obtained for hexadec-9-enoic acid-9,10-d_2. Note that for this experiment the number of samples for the substrate group was 58.

Looking at the data shown in the boxplots, there does appear to be some differences in the median percentage abundances and range of the data. For both $I^{(M+1)}$ and $I^{(M+2)}$ the median value for pentacosene-2 is greater for the substrate data than for the control data. The results of the Mann-Whitney U-tests reveal the statistical differences.

The results show that there is statistical significance for pentacosene-2 at both $I^{(M+1)}$ and $I^{(M+2)}$, with p values of 0.041 and 0.016 respectively. Although the value for

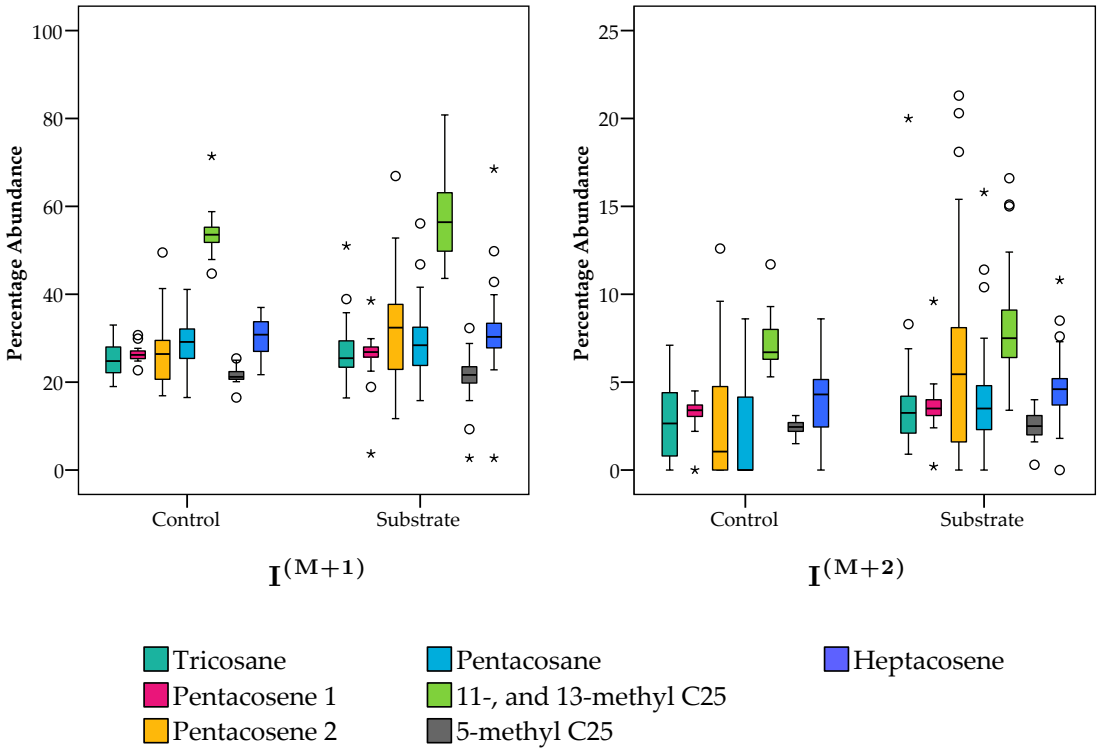


Figure 6.14: Boxplots showing the data for hexadec-9-enoic acid-9,10-d_2. The data for $I^{(M+1)}$ is on the left and $I^{(M+2)}$ on the right.

$I^{(M+1)}$ is only just below the critical value of p (0.050) it still indicates a difference in the groups. It is expected that this value would be higher than that for $I^{(M+2)}$ as each incorporated molecule of labelled fatty acid should incorporate two extra mass units from the deuterium labels. Therefore each resulting alkene should be two mass units heavier than the equivalent control. Therefore in theory there should be no statistical difference between the two groups for this compound at the $I^{(M+1)}$ level, however this can be explained through hydrogen exchange. During this process one of the labels is lost and replaced with a hydrogen atom. This yields an alkene with just one remaining label and this molecule is therefore just 1 Da heavier.

Despite its prevalence in nature palmitoleic acid, or hexa-9-decenoic acid, is a ω-7 fatty acid and thus it would be used in the biosynthesis of a Z7 alkene compound. According to Table 3.1 this is not a positional isomer found in *Myrmica sabuleti*, but it is found in *Myrmica scabrinodis*. This may explain why the incorporation of this compound was in very small amounts as it is not a compound that would normally be biosynthesised

Table 6.7: The probability values from the calculated Mann-Whitney U-tests for hexadec-7-enoic acid-7,8-d_2. Note that any p values less than 0.050, and therefore significant, are underlined for ease of reference.

Compound	p value	
	Hexadec-9-enoic acid-9,10-d_2	
	$I^{(M+1)}$	$I^{(M+2)}$
Tricosane	0.252	0.145
Pentacosene-1	0.136	0.188
Pentacosene-2	<u>0.041</u>	<u>0.016</u>
Pentacosane	0.476	<u>0.012</u>
11-, and 13-methyl C25	0.057	0.178
5-methyl C25	0.449	0.305
Heptacosene	0.398	0.135

by this species. Nevertheless there is evidence that the labelled compound is being used to biosynthesis an alkene, however the nature of the resulting compound has not been characterised.

The Mann-Whitney U-tests also indicate that once again significance is indicated for pentacosane at the $I^{(M+2)}$ level, continuing the trend that has so far been observed for the majority of the tested substrates. One theory for this continued statistical significance could be the control data as this is consistent throughout this experiment, each substrate is tested against the same set of control values. Therefore if the control values for pentacosane were unusually low then this could make all the substrate data appear high. As previously described in Section 3.4.2.1 there is an equation to calculate the expected values for $I^{(M+2)}$. This equation can be used to check the quality of the control data to ensure that none of the values are too low and thus misrepresentative of a control. As Equation 3.3 states the value for $I^{(M+2)}$ is calculated by multiplying the square of the number of carbons in the compound by 0.006. In this case the theoretical value for pentacosane and $I^{(M+2)}$ is 3.75%. This is greater than the experimentally obtained control value of 3.1%, the issue with this is that a lower comparative control value makes the substrate data seem higher. This perhaps explains why this trend is

being seen across the data. Further analysis of this issue will be covered at the end of the chapter.

6.3.8 Hexadec-11-enoic acid-11,12-d_2

This was the final fatty acid with sixteen carbons in the chain. Hexadec-11-enoic acid-11,12-d_2 is also known as palmitvaccenic acid and is an uncommon fatty acid found in bacterial and animal lipids [12]. For this experiment n=38. The following data refers to the data obtained for hexadec-11-enoic acid-11,12-d_2.

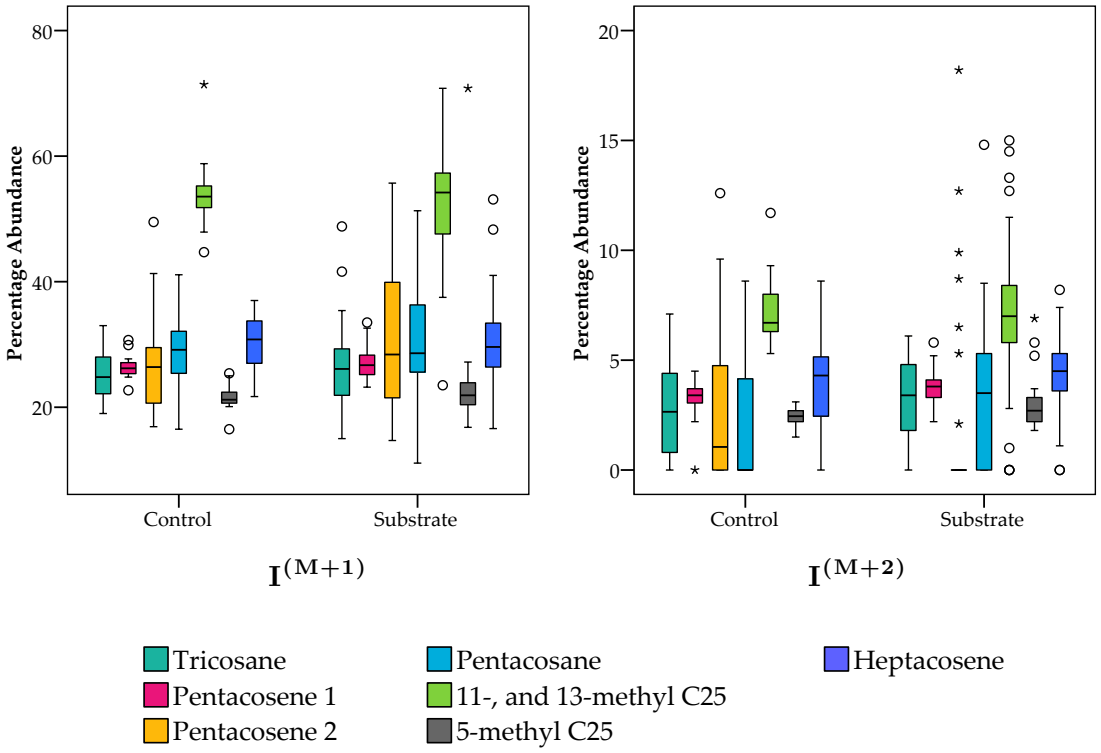


Figure 6.15: Boxplots showing the data for hexadec-11-enoic acid-11,12-d_2. The data for $I^{(M+1)}$ is on the left and $I^{(M+2)}$ on the right.

The boxplots show that there are few differences in the medians between the groups, see Figure 6.15. There are however bigger differences in the ranges of the data with a general trend that the substrate data has a greater range with far more outliers. This is to be expected and may be indicative of a few individual ants showing a trend that

is not shown at a group level.

In order to determine statistical significance, between the groups for any of the studied compounds, Mann-Whitney U-tests were performed. Please see Table 6.8 for the results of this statistical analysis.

Table 6.8: The probability values from the calculated Mann-Whitney U-tests for hexadec-11-enoic acid-11,12-d_2. Note that any p values less than 0.050, and therefore significant, are underlined for ease of reference.

Compound	p value	
	Hexadec-11-enoic acid-11,12-d_2	
	$I^{(M+1)}$	$I^{(M+2)}$
Tricosane	0.318	0.120
Pentacosene-1	0.315	<u>0.050</u>
Pentacosene-2	0.113	*0.017*
Pentacosane	0.295	0.121
11-, and 13-methyl C25	0.341	0.407
5-methyl C25	0.338	<u>0.049</u>
Heptacosene	0.453	0.157

The results of the Mann-Whitney U-tests indicate that there is no significant difference for any of the compounds at the $I^{(M+1)}$ level. At the $I^{(M+2)}$ level there is technically significance shown for the minor isomer of pentacosene and 5-methylpentacosane. However it is worth looking at the p values. The p value for 5-methylpentacosane is 0.049; whilst this is a significant result it is only just below the significance level (≤ 0.050). It is worth considering that this result is based on analysis of the raw data and the chromatograms. This is a process which is inherently subjective, and although every effort was taken to ensure that it was a standardised process it was not always possible. Therefore a value which is just under the critical value for p may not be truly indicative of statistical significance.

Given what is known about about the biosynthesis of hydrocarbons it is highly unlikely

that hexadec-11-enoic acid-11,12-d_2 could be used in the biosynthesis of a methyl-branched compound. Methyl-branched compounds are known to be biosynthesised using the *de novo* synthesis of the carbon chain using acetates, and when necessary, a propionate molecule to introduce the methyl branch. Therefore it could be assumed that the indicated incorporation shown here for 5-methylpentacosane is erroneous. However the incorporation of an extra mass was previously observed for 5-methyl pentacosane at the $I^{(M+1)}$ level for tetradec-9-enoic acid-9,10-d_2. Additional incorporation observed here suggests that this may not be an anomalous result, possible explanations are explored later on in the chapter.

Hexadecenoic acid overview

At this point it is worth considering all of the hexadecenoic acid substrates as a whole. In total five different substrates were tested. Looking at the results of these substrates, there do not appear to be trends occurring across them. As was seen for the tetradecenoic acid substrates, some show incorporation for pentacosane at the $I^{(M+2)}$ level, but not all. Out of the five tested substrates of this type, two provided interesting results; hexadec-5-enoic acid-5,6-d_2 and hexadec-9-enoic acid-9,10-d_2. Although other substrates showed incorporation for various compounds at various levels these were the only two to show positive incorporation at both studied levels for alkenes. If an unsaturated fatty acid compound is successfully incorporated it is expected that this would be seen at both levels (although predominantly at the $I^{(M+2)}$ level) and primarily within alkenes. Of the five studied substrates only two showed these attributes. Hexadec-5-enoic acid-5,6-d_2 showed incorporation for the primary pentacosene isomer, whilst hexadec-9-enoic acid-9,10-d_2 showed incorporation for the secondary isomer.

The possible reasons for the incorporation of these substrates for alkene compounds are discussed within each individual section, however it is likely that the ω-type of the fatty acid is important in precursor selection. Hexadec-5-enoic acid-5,6-d_2 is a is a ω-11 fatty acid and would thus produce a Z11 isomer, one that is found in *Myrmica*

sabuleti, although it is a minor isomer of the species. Hexadec-9-enoic acid-9,10-d_2 is a ω-7 fatty acid and thus it would be used in the biosynthesis of a Z7 alkene compound. However this is not a positional isomer found in *Myrmica sabuleti* so it is not known why incorporation is seen for this compound.

6.3.9 Octadec-7-enoic acid-7,8-d_2

The next series of labelled fatty acids to be tested were those containing 18 carbons. Octadec-7-enoic acid-7,8-d_2 is an ω-11 acid, however it is not found in nature. For this experiment n=74. The following data refers to that obtained for the labelled substrate octadec-7-enoic acid-7,8-d_2.

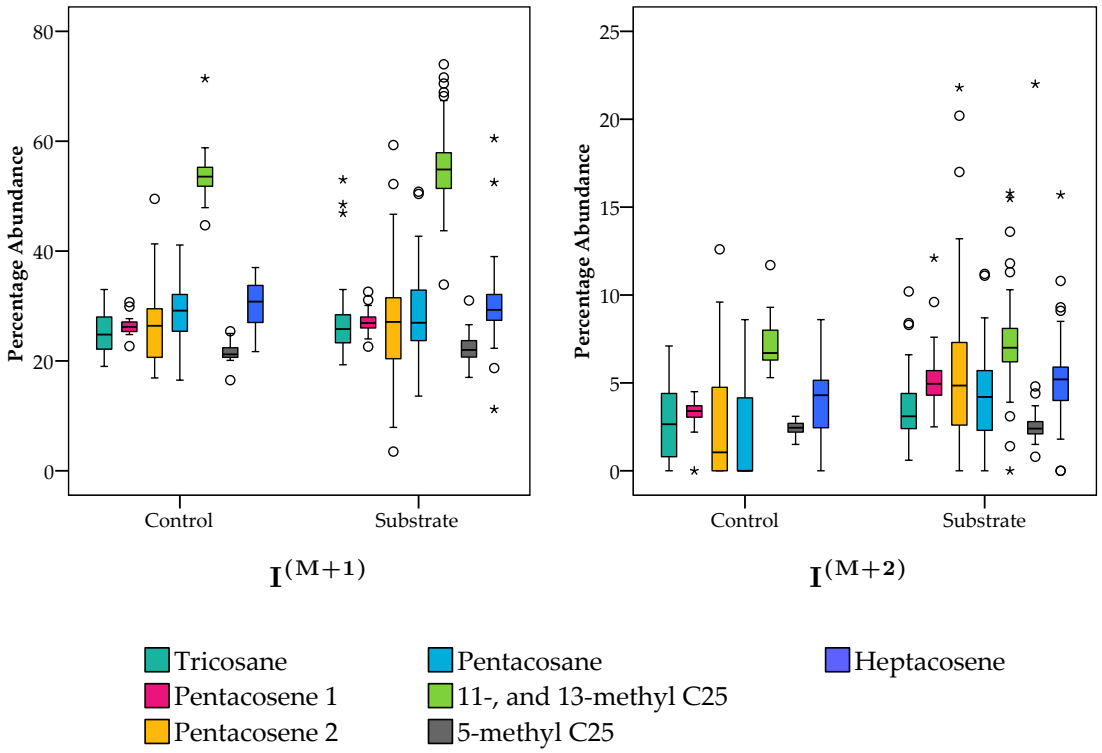


Figure 6.16: Boxplots showing the data for octadec-7-enoic acid-7,8-d_2. The data for $I^{(M+1)}$ is on the left and $I^{(M+2)}$ on the right.

Looking at the data shown in Figure 6.16 it can be seen that the results for $I^{(M+1)}$ are similar between groups. However there is a much greater difference in the boxplot for $I^{(M+2)}$. Within this plot it can be seen that for several compounds the median percent-

age abundance is greater in the substrate group compared to the control group. There are however far more outliers shown within the substrate data. In order to quantify statistical differences Mann-Whitney U-tests were performed on this data set. Table 6.9 shows the results of this analysis.

Table 6.9: The probability values from the calculated Mann-Whitney U-tests for octadec-7-enoic acid-7,8-d_2. Note that any p values less than 0.050, and therefore significant, are underlined for ease of reference.

Compound	p value	
	Octadec-7-enoic acid-7,8-d_2	
	$I^{(M+1)}$	$I^{(M+2)}$
Tricosane	0.198	0.059
Pentacosene-1	<u>0.048</u>	<u>0.000</u>
Pentacosene-2	0.432	<u>0.005</u>
Pentacosane	0.370	<u>0.002</u>
11-, and 13-methyl C25	0.155	0.463
5-methyl C25	0.231	0.479
Heptacosene	0.257	<u>0.003</u>

The results of the U-tests reveal that there are several compounds that are statistically significant between the groups at the $I^{(M+2)}$ level, but only one which is significant at the $I^{(M+1)}$ level. This compound is the major isomer of pentacosene, however the p value associated with this compound is 0.048 and therefore it can be considered to be only just significant at this confidence level. As has been previously discussed the way in which the data is processed can be subjective and therefore values which are close to the critical value of p are less meaningful than those which are closer to 0.000.

There are several compounds significant at the $I^{(M+2)}$ level. Once again significance is seen for pentacosane, this could be due to the use of this fatty acid and subsequent hydrogenation to biosynthesise a saturated alkane from an unsaturated fatty acid. Whilst this might seem energetically unfavourable there would be a large supply of this precursor available, and it may be that this choice is driven by availability of the

precursor. However as previously discussed the significant results observed for many of the substrates and this compound at the $I^{(M+2)}$ may be due to the quality of the control data.

There are however three alkene compounds which are showing statistical significance for the $I^{(M+2)}$ level. This incorporation makes more biological sense as it is logical that an unsaturated fatty acid would yield an unsaturated hydrocarbon. Octadec-7-enoic acid-7,8-d_2 is an ω-11 acid and, as shown in Table 3.1, *Myrmica sabuleti* is known to produce the Z11 isomer of pentacosene, albeit in small amounts [16]. Based on this it is entirely possible that these ants are using this labelled fatty acid to biosynthesise the Z11 isomer of pentacosene. However incorporation of this fatty acid is also seen for the major isomer of pentacosene as well as the minor isomer. It could be that this is down to the way in which the data is processed. The positions of the major and minor isomers are predicted based on retention times, and the ion counts taken from these predicted areas. Therefore there may be some overlap of the peaks when they are not distinct as shown in Figure 6.6. This may make it look like the labelled compound is appearing in other isomers of pentacosene when it is in fact only present in the Z11 compound. However in order to confirm or refute this extra analysis would have to be performed on these samples.

From previous research done on the positional isomers of the alkenes biosynthesised by *Myrmica sabuleti* it is known that they produce the Z11 isomer of pentacosene, however analysis did not reveal the presence of the Z11 isomer of heptacosene. Statistical analysis indicates that the labelled fatty acid is incorporated into heptacosene. Again this could be that availability of a precursor compound drives a change in the isomers biosynthesised, even if this means that the ants are producing a compound not normally found within this species.

This fatty acid is not normally found in nature, therefore if this fatty acid is recog-

nised as a suitable precursor for alkene biosynthesis then it must be because it has the potential to be biosynthesised *de novo* by these ants. This may explain why it is only a minor isomer found within this species as a natural precursor does not exist, making the formation of a Z11 alkene less biologically favourable than say a Z9 equivalent. The Z9 positional isomer would be formed from a ω-9 acid of which the most prevalent is oleic acid, found widely in nature and natural sources.

6.3.10 Octadec-11-enoic acid-11,12-d_2

The second of the labelled fatty acids containing 18 carbons to be tested was octadec-11-enoic acid-11,12-d_2. This compound is widely known by its common name cis-vaccenic acid. Although fairly uncommon in nature it is found in the fruit of the sea buckthorn plant [17]. For this substrate the number of samples was 70. The following data refers to that obtained for the labelled substrate octadec-11-enoic acid-11,12-d_2. Please see Figure 6.17 for the boxplots.

These boxplots show several differences between the data for the substrate group and the data for the control group. The median percentage abundance for the secondary pentacosene isomer is much greater within the substrate group compared to that of the control group, for both studied levels. However, in contrast to data seen up to this point, the range of the data is still small, indicating consistency within the results across the dataset. The difference between the groups for pentacosene-2 at the $I^{(M+2)}$ level is remarkable with average percentage abundance values of 353% for the substrate compared to 3.1% for the control. Please refer to Appendices D.21-D.23 for the raw data, which clearly shows the very high percentage abundance values for pentacosene-2 at the $I^{(M+2)}$ level. As the raw data suggests there was a clear difference in the appearance of the mass spectra between the two groups for the compound pentacosene-2. For a schematic showing the comparable mass spectral data see Figure6.18.

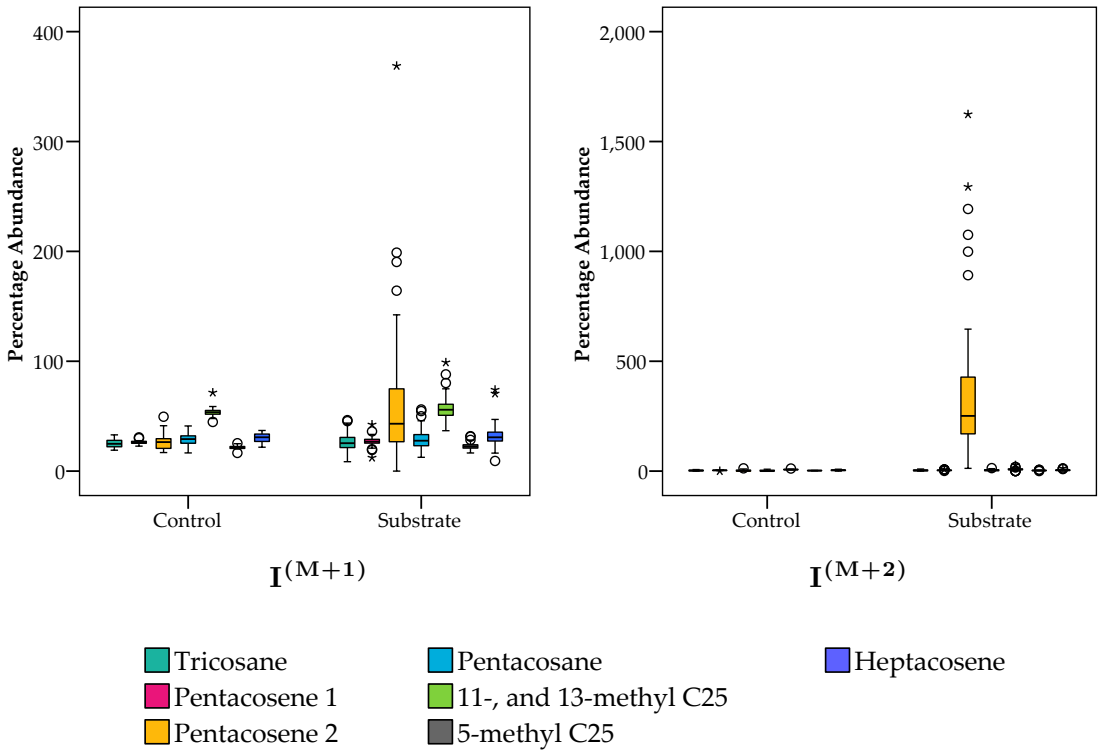


Figure 6.17: Boxplots showing the data for octadec-11-enoic acid-11,12-d_2. The data for $I^{(M+1)}$ is on the left and $I^{(M+2)}$ on the right.

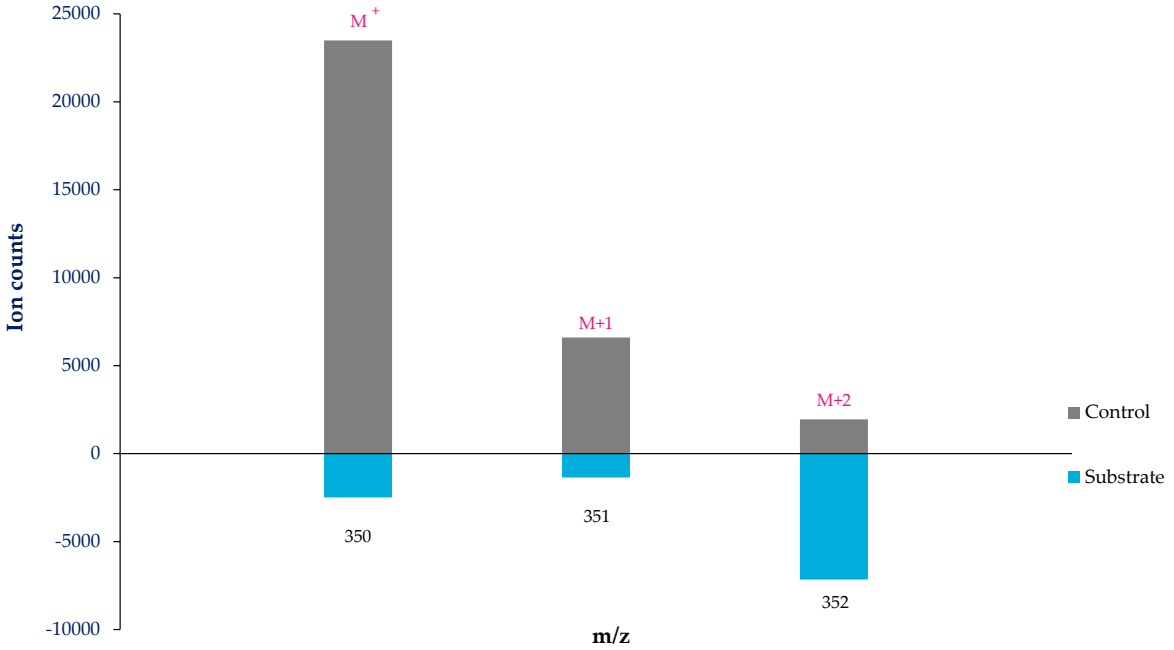


Figure 6.18: Extracted mass spectrum for the octadec-11-enoic acid-11,12-d_2 experiment showing the average comparative ion counts for control and substrate data sets for the compound pentacosene-2.

Generally the substrate mass spectra showed ion counts that were much greater for the M+2 isotope (*m/z* 352) compared to the molecular ion [$M^{+\bullet}$] (*m/z 350*). The control group on the other hand showed a 'normal' mass spectrum appearance with a gradual decrease in the ion counts for the various iostopes. This difference can be seen in Figure 6.18 which represents the average ion counts for the substrate and control group for the compound pentacosene-2. The other compounds showed ion counts that followed the expected trends and hence they are not shown here.

In order to further analyse the differences between the experimental groups, Mann Whitney U-tests were performed on this data to determine the statistically significant compounds.

Table 6.10: The probability values from the calculated Mann-Whitney U-tests for octadec-11-enoic acid-11,12-d_2. Note that any p values less than 0.050, and therefore significant, are underlined for ease of reference.

Compound	p value	
	Octadec-11-enoic acid-11,12-d_2	
	$I^{(M+1)}$	$I^{(M+2)}$
Tricosane	0.290	0.165
Pentacosene-1	0.145	0.418
Pentacosene-2	<u>0.000</u>	<u>0.000</u>
Pentacosane	0.452	<u>0.010</u>
11-, and 13-methyl C25	0.114	0.093
5-methyl C25	0.093	<u>0.024</u>
Heptacosene	0.327	0.502

The results shown in Table 6.10 indicate that there are several compounds which are statistically significant between the substrate group and the control group. Once again significance is indicated for pentacosane but only at the $I^{(M+2)}$ level. However significance is also indicated for the methyl-branched compound 5-methylpentacosane. As previously discussed, the route of synthesis of this compound would be via a *de novo* fatty acid synthesis using acetate and propionate molecules, resulting in a fatty acid

which contains a methyl branch. As this result has been observed several times it may be that there is a different process leading to incorporation. One possible suggestion is that the incorporation shown here is down to a metabolic process and subsequent usage of the breakdown products. This theory will be discussed in more detail at the end of the chapter.

The main finding of this experiment is the clear incorporation of the fatty acid into a secondary alkene isomer. When analysing the chromatograms for this substrate it was clear that there was a notable difference in the appearance of the chromatograms for the substrate group and hence the chemical profile of the samples. The extractions from the substrate group showed a clear shoulder to the main pentacosene peak, as shown in Figure 6.6. The height of this peak varied in intensity but was clearly defined in the majority of the substrate chromatograms. This shoulder was not present within the chromatograms of the control samples. It was the presence of this extra peak which prompted the other substrates to be re-analysed, in case this was happening with the other substrates, albeit it in a more subtle way.

The results of this experiment indicate that there is clear incorporation of the labelled fatty acid octadec-11-enoic acid-11,12-d_2 into a pentacosene isomer. In addition the the results indicate that when *Myrmica sabuleti* are fed a diet containing this labelled compound the ants use it to biosynthesise a specific pentacosene isomer, which is not normally produced by this species, as evidenced by the extra shoulder present on the pentacosene peak. However there is also statistical significance shown at the $I^{(M+1)}$ level. This indicates that sometimes a molecule of the pentacosene isomer is produced with only one deuterium label present and hence only 1 Da heavier than the unlabelled equivalent. This suggests that sometimes only one deuterium label survives the biosynthetic modifications intact. This is possibly caused by the exchange of a deuterium atom for a hydrogen atom during the biosynthetic process. However it should also be noted that this is more likely to be caused by incomplete labelling during the synthesis

of these compounds. Although the method used to add the labels should have resulted in two deuteriums being added across the double bond, in reality this would be impossible to achieve as this will ultimately be dependant on the purity of the deuterium gas, and the deuteration process.

In order to further understand the processes that were occurring, and specifically which isomer was biosynthesised using this substrate, a standard dimethyl disulfide reaction was carried out. See Section 6.2.1 for details of the method that was used. As previously discussed this reaction involves the addition of two CH_3S groups across the double bond within an alkene molecule. When this molecule undergoes fragmentation in the mass spectrometer the double bond position is easily identified since cleavage occurs at the carbon-carbon bond between the CH_3S groups. This causes the formation of two main diagnostic ions, represented by $A^{(+)}$ and $B^{(+)}$ in Figure 6.19 below [6].

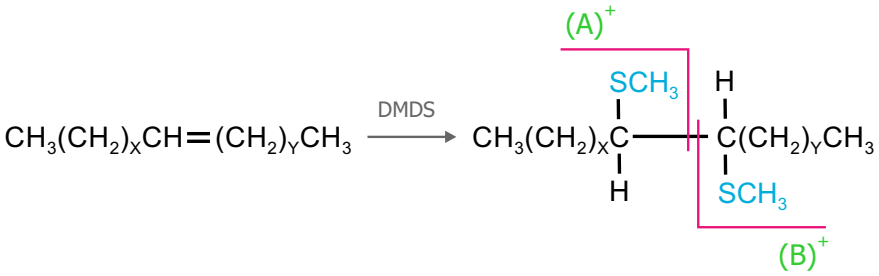


Figure 6.19: A schematic of the DMDS reaction of an alkene, based on Carlson, [6].

This fragmentation and the resulting formation of two fragment ion leads to characteristic mass spectra, the correct interpretation of which provides the double bond positions of a particular isomer, please see Figure 6.20 for reference. Figure 6.20 shows an example fragmentation pattern after DMDS analysis has been performed on heptadecene. The identity of the compound can be determined by taking the molecular weight of the resulting adduct, in this case 332 m/z, and subtracting 94, which is the mass of the two SMe groups. This answer is then divided by 14 to reveal the length of the carbon chain, in this case 17. The position of the double bond can be identified

by taking the smaller of the two fragment masses, subtracting 47, which is the mass of one SMe group and dividing this answer by 14. In the example shown in Figure 6.20 the double bond position indicated is 8.

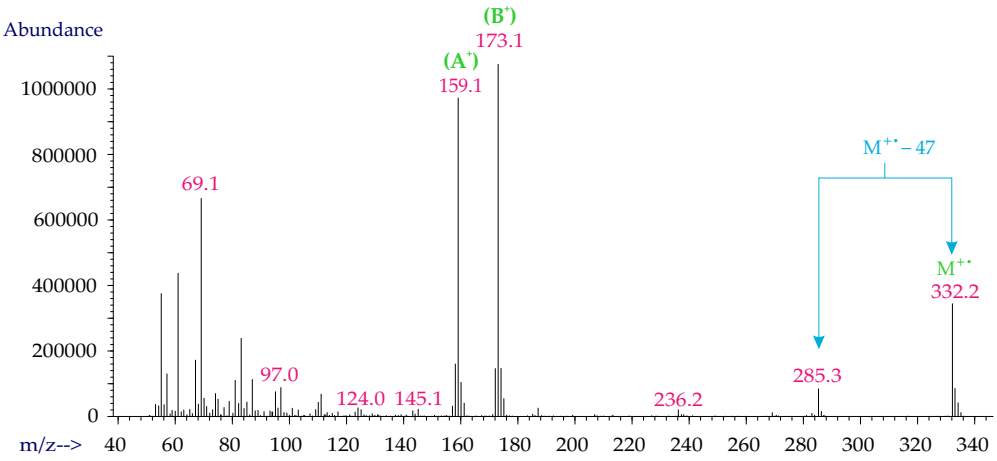


Figure 6.20: An example DMDS mass spectrum. The masses of $A^{(+)}$ and $B^{(+)}$ equal the molecular weight of the derivatised compound, in this case heptadecene (m/z 332) whilst the masses of the fragments indicate the double bond position (Z8).

The results from this experiment revealed that the ants within the substrate group which were fed octadec-11-enoic acid-11,12-d_2 were biosynthesising a different pentacosene isomer to that found in the control samples. Therefore this DMDS reaction was used to determine the position of all the double bonds within the pentacosene isomers for the substrate and control ants. In particular it was hoped that the results of this reaction would indicate the isomer position that was being biosynthesised using the labelled precursors.

Figure 6.21 shows an example mass spectra from one of the control sample pooled extracts. This spectrum is from 19.336 minutes, and as indicated the three sets of fragment ions show the three pentacosene isomers which can be clearly identified.

As Figure 6.21 indicates the ants which were fed a standard diet (no chemical sub-

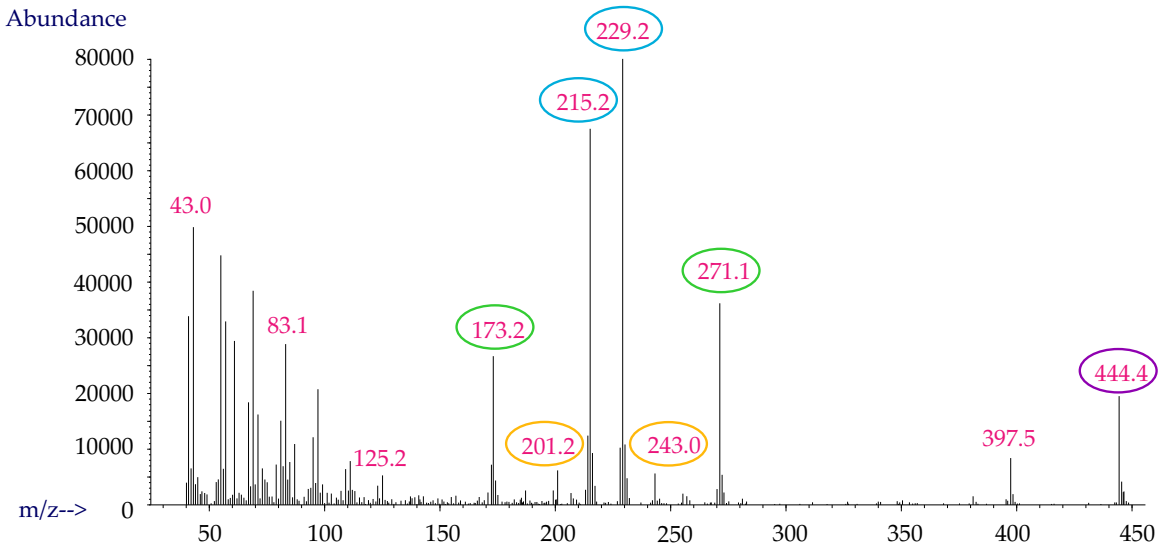


Figure 6.21: The resulting DMDS mass spectrum from the control fed *Myrmica sabuleti* ants; the ion pairs in green, yellow and blue respectively indicate Z9, Z11 and Z12 isomers of pentacosene.

strate) have a mixture of pentacosene isomers, indicating that these ants will normally synthesis pentacosene molecules with a potential variety of double bond positions. It is not known what influences these positions, nor why these are the one that are found, however the data from these control ants and the double bonds that were determined match the previous research done on this species. This is both as part of this study, as shown in Table 3.1, and in other research studies such as that in 2012 by Guillem et al. [16]. In this study the author looked at the chemical profiles of two morphologically similar ants; *Myrmica sabuleti* and *Myrmica scabrinodis*. In the aforementioned study it was found that *Myrmica sabuleti* produced pentacosene with double bonds in the Z9, Z11 and Z12 positions. This supports the findings of the DMDS reaction that was carried out for this project and therefore suggests that these positions are relatively stable and species-specific.

The DMDS mass spectrum in Figure 6.22 shows the results from one of the pooled extracts of 20 ants fed on a substrate diet consisting of deuterium labelled octadec-11-enoic acid-11,12-d_2. Each DMDS adduct appears at a slightly different point in the

chromatogram as the retention time varies very slightly. However these peaks tend to overlap, therefore different mass spectra are acquired by clicking on differing areas of the peak associated with the pentacosene adducts. This mass spectra is obtained at 19.336 minutes, to allow for direct comparison with the control. This mass spectrum clearly shows the elution of two different adducts resulting from the different isomers of pentacosene.

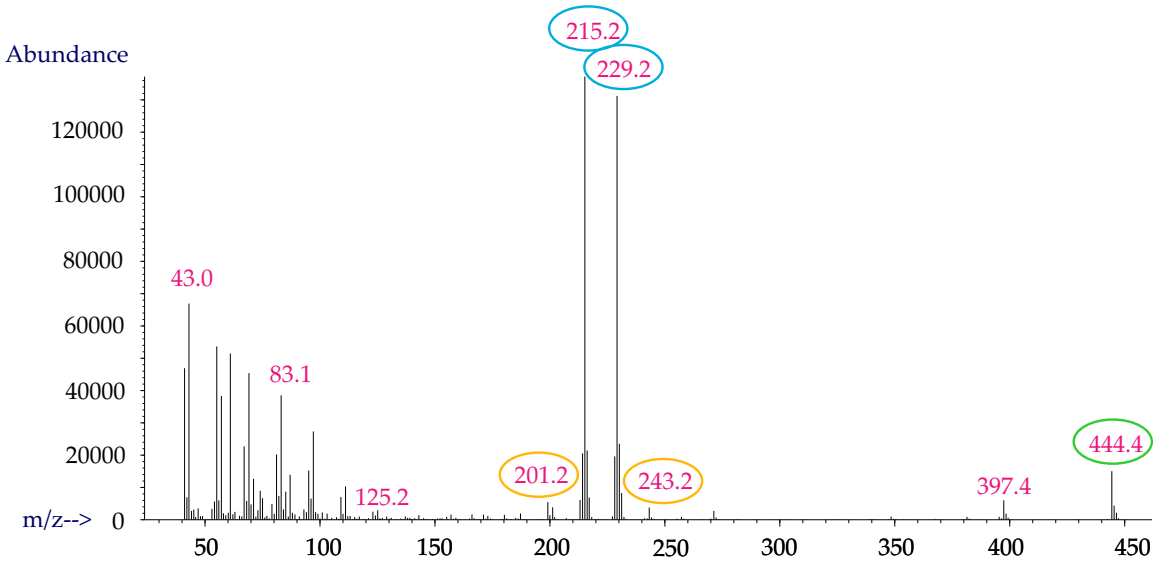


Figure 6.22: A resulting DMDS mass spectrum from the octadec-11-enoic acid-11,12-d_2 fed ants, taken from 19.344 minutes; again indicating Z11 and Z12 pentacosene isomers.

Figure 6.22 shows that the observed double bond positions are slightly different compared to the control example shown in Figure 6.21. Specifically that there is very little Z9 adduct in the substrate example. Note that this is such a minor isomer that whilst present, it is not indicated in Figure 6.22. The majority of the isomers shown are either Z11 (yellow) and Z12 (blue). The ion pairs associated with these adducts have a molecular weight of 444 *m/z* (indicated by a pink oval). This is because a DMDS reaction adds two SMe groups across the double bond, and each contributes a mass of 47 units, therefore the total weight of the DMDS product is 350+94; 444 *m/z*. However this mass indicates that there are no deuterium labels incorporated into the mass

adducts, therefore the labelled substrate was not used to biosynthesise either of these isomers.

In the same way Figure 6.23 shows a mass spectrum taken from the same pooled substrate extract as that in Figure 6.22, however this spectrum is taken from slightly later in the chromatogram at 19.378 minutes. It can be seen that within this spectrum there are some differences to that shown in Figure 6.22.

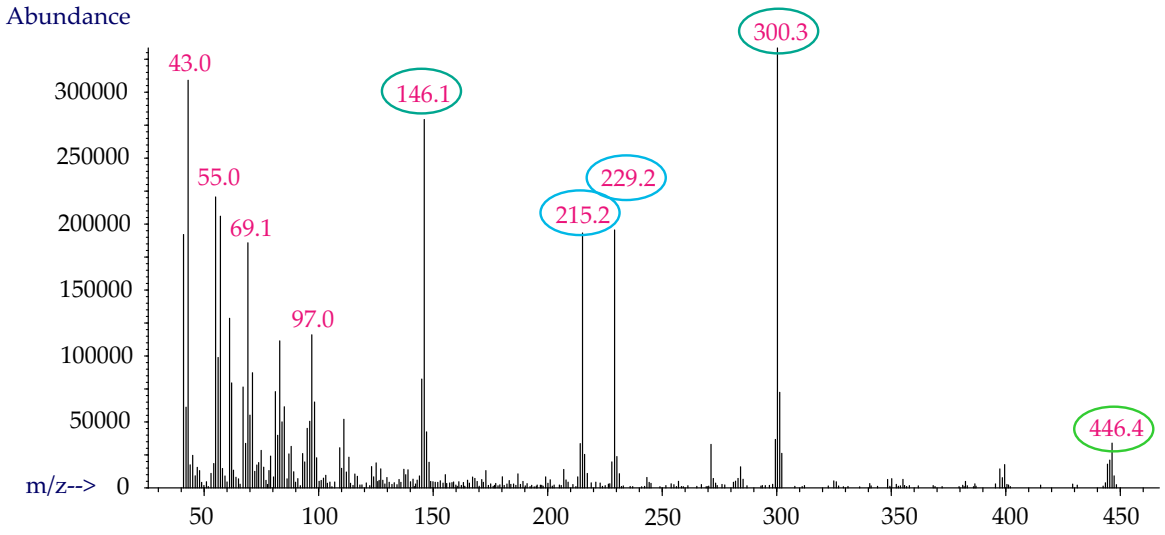


Figure 6.23: A resulting DMDS mass spectrum from the octadec-11-enoic acid-11,12-d_2 fed ants, taken from 19.378 minutes; this time indicating Z7 and Z12 pentacosene isomers (turquoise and blue respectively).

As can be seen from Figure 6.23 there are two sets of fragment ions indicated in blue and turquoise. The total masses of these fragment ions is different, with the blue pair totalling 444 *m/z* and the turquoise totalling 446 *m/z*, an increase of two mass units. The increase of two mass units corresponds to the addition of two deuterium atoms across the double bond, as shown in Figure 6.24. The turquoise ion pair in this adduct are 146 *m/z* and 300 *m/z*, these indicate that a double bond is present on the seventh carbon and therefore a Z7 isomer of pentacosene is present. This is an interesting result as the control ants do not show this double bond position, even in low concentrations.

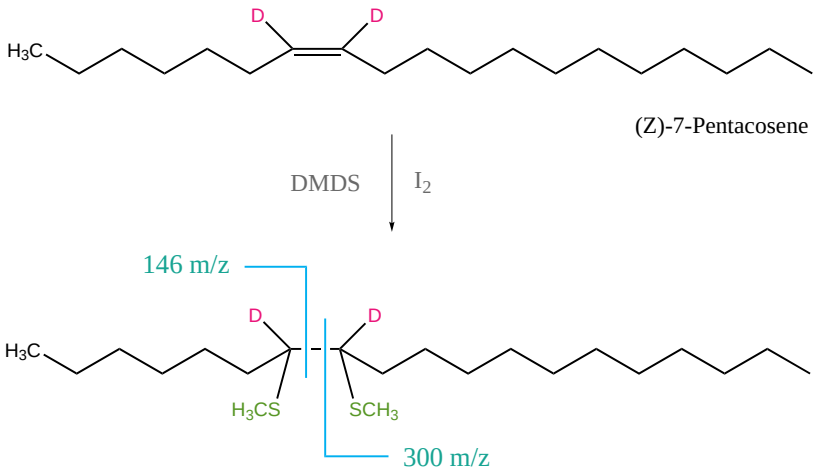


Figure 6.24: DMDS reaction scheme which shows the formation of the newly observed ions and their relative masses resulting from Z7 pentacosene.

As previously described the nomenclature of fatty acids is such that the numbering of carbons begins from the carboxylic acid group, hence octadec-11-enoic acid-11,12-d_2, has the double bond between the 11th and 12th carbons (Z11 position). However for alkenes, the nomenclature is such that the carbons are numbered so that the double bond position is in the lowest numerical location. Hence when octadec-11-enoic acid-11,12-d_2 is used to make an alkene the double bond resulting from this compound is in the Z7 position when the final pentacosene molecule is synthesised. See Figure 6.25 for clarification.

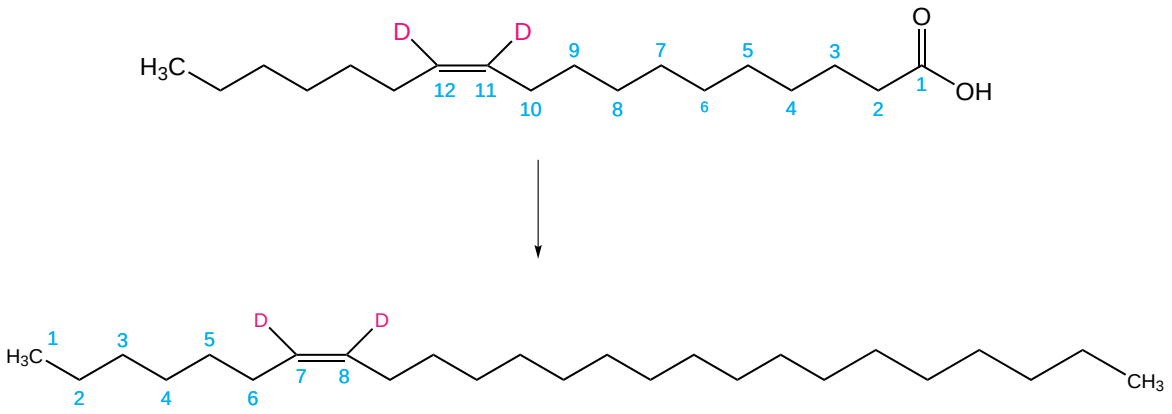


Figure 6.25: Schematic showing the nomenclature and carbon numbering protocol of C18 Z11, and how this results in a Z7 alkene.

The control group ants that were chemically analysed did not show the presence of a Z7 isomer, and it is not a double bond position that is usually found in *Myrmica sabuleti*. Instead the isomers generally detected are Z9, Z11, or Z12. However when fed octadec-11-enoic acid-11,12-d_2, a substrate which contained a double bond in an equivalent Z7 position, the ants were able to use this to make the corresponding (Z)-7-pentacosene isomer. This suggests that the ants use the precursor as it is, recognising that it is suitable for use, and not making any modifications to it. However as previously mentioned this is not an isomeric position that is usually found within this species. Its presence at the end of this experiment may suggest that the recognition of this as a suitable precursor and the subsequent enzymes for its use might belong to a mechanism that is still present but not normally active.

A closely related species to that of *Myrmica sabuleti* is *Myrmica scabrinodis*. In the same study by Guillem in 2012 the isomeric forms of pentacosene were also identified for this species. Their study found that *Myrmica scabrinodis* produced Z9 and Z12 isomers [16], however the results of the initial characterisation of this species presented in Table 3.1 showed that *Myrmica scabrinodis* also produce the Z7 isomer of pentacosene in reasonable amounts. Therefore *Myrmica scabrinodis* are able to routinely biosynthesise a Z7 isomer of pentacosene. According to a study by Pech [18] *Myrmica*

sabuleti, *Myrmica scabrinodis*, and *Myrmica hirsuta*, all belong to the same clade and as such are sister species, see Figure 6.26 which shows a simplified cladogram of the most common *Myrmica* species. Whilst this figure shows only 14 species within the *Myrmica* genus, there are over 200 known.

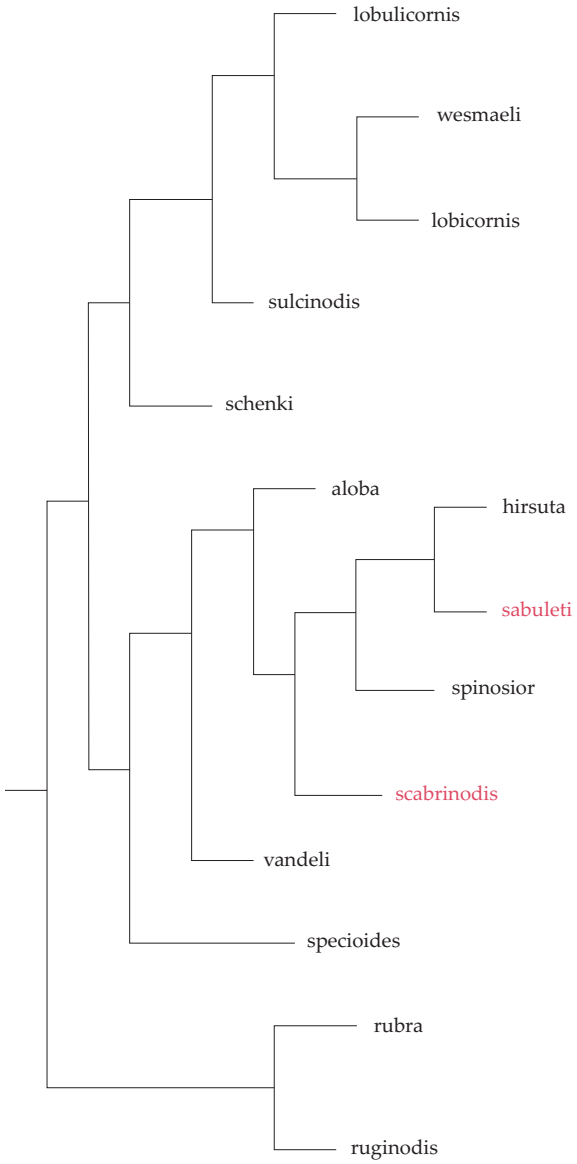


Figure 6.26: A simplified cladogram of twelve *Myrmica* species indicating the relationship between *M. sabuleti* and *M. scabrinodis*, adapted from [20].

In 2010 a study by Jansen et al. estimated the diversion times of the species within the *Myrmica* genus and found that *Myrmica scabrinodis* diverged from their most closely

related species, including *Myrmica sabuleti*, approximately 4 million years ago [19]. Given that these species are genetically linked it is possible, although certainly not yet proven, that there could be some sharing of biosynthetic characteristics and pathways between these highly related species. This may mean that the biosynthetic pathway to produce a Z7 compound is present in several of these sister species but only active in some. Therefore *Myrmica sabuleti* could also have the ability to synthesis Z7 compounds even though they do not routinely produce this compound. This may be so that the chemical profile of each species is distinct and that diversification has evolved so that individuals can identify between species. It should be stressed that this is a very tentative hypothesis, and whilst there is no firm supporting evidence, other than that presented above, there is no doubt that when fed octadec-11-enoic acid-11,12-d_2 *Myrmica sabuleti* are able to use it in order to biosynthesis a Z7 pentacosene isomer, a position which is not normally found within this species.

Although *Myrmica scabrinodis* and *Myrmica sabuleti* appear very morphologically similar they do feature distinct chemical profiles. Both *Myrmica scabrinodis* and *Myrmica sabuleti* can be separated from other British *Myrmica* species by looking at the overall chemical profile of their cuticular hydrocarbons. Unlike other *Myrmica* species *M. scabrinodis* and *M. sabuleti* are rich in pentacosene isomers. The main difference in cuticular hydrocarbons between the two species is that *M. scabrinodis* shows high levels of 3-methyltricosane, whilst *M. sabuleti* shows high levels of 5-methylpentacosane. All ants analysed during the course of this experiment showed levels of 5-methylpentacosane that were consistent with this species, which acts as a useful marker in helping to distinguish between the species. Therefore it cannot be suggested that these results are due to initial misidentification of the species.

6.3.11 Eicos-9-enoic acid-9,10-d_2

The next substrates to be tested were those with the longest carbons chains. The first of the two was Eicos-9-enoic acid-9,10-d_2. Also known as gadoleic acid, this fatty acid is found in the triglycerides of fish oils [12]. For this experiment the number of substrate samples analysed was 33. The following data refers to that obtained for the labelled substrate Eicos-9-enoic acid-9,10-d_2, see Figure 6.27 for the boxplots.

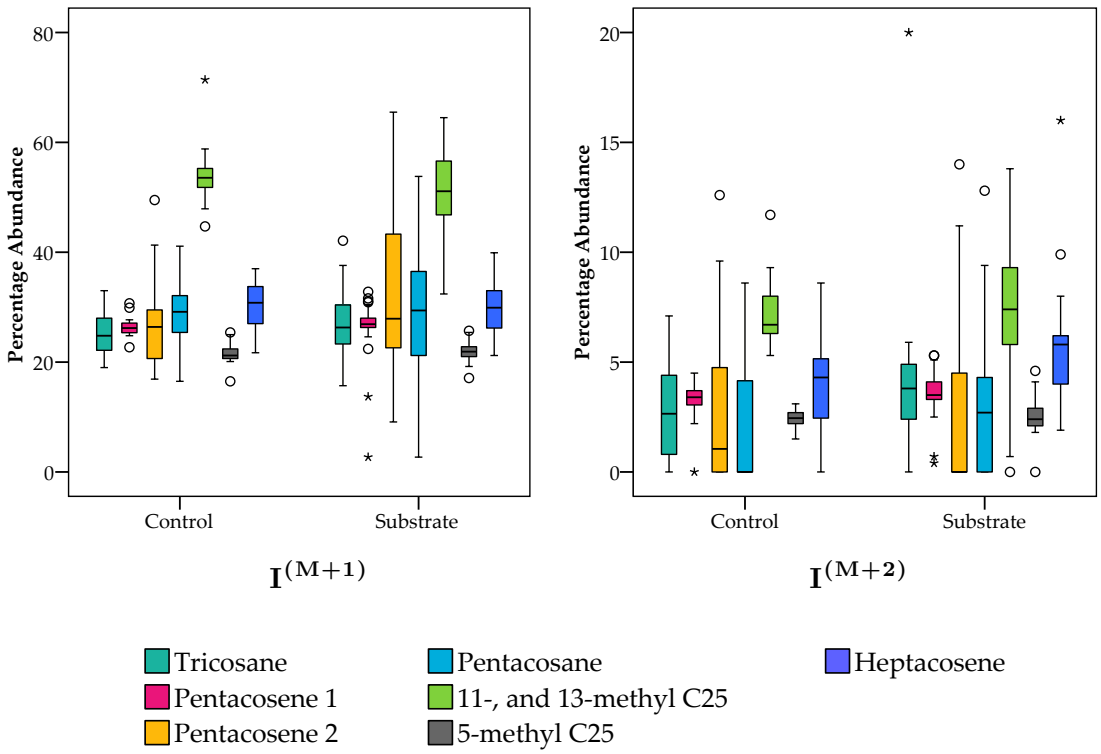


Figure 6.27: Boxplots showing the data for Eicos-9-enoic acid-9,10-d_2. The data for $I^{(M+1)}$ is on the left and $I^{(M+2)}$ on the right.

Figure 6.27 shows that there is very little difference in the median percentage abundances for the two groups. The range of the data is also fairly similar between the groups. The results of the Mann-Whitney U-tests are shown in Table 6.11.

As can be seen from Table 6.11 there is only one compound which is statistically significant between groups; heptacosene at the $I^{(M+2)}$ level. This compound has a p value of 0.005. Eicos-9-enoic acid-9,10-d_2 is a ω-11 acid and would therefore be biosynthet-

Table 6.11: The probability values from the calculated Mann-Whitney U-tests for Eicos-9-enoic acid-9,10-d_2. Note that any p values less than 0.050, and therefore significant, are underlined for ease of reference.

Compound	p value	
	Eicos-9-enoic acid-9,10-d_2	
	$I^{(M+1)}$	$I^{(M+2)}$
Tricosane	0.113	0.064
Pentacosene-1	0.053	0.158
Pentacosene-2	0.148	0.169
Pentacosane	0.232	0.294
11-, and 13-methyl C25	0.161	0.269
5-methyl C25	0.185	0.447
Heptacosene	0.380	<u>0.005</u>

ically used to produce a Z11 alkene. Based on previous characterisation studies it is known that *Myrmica sabuleti* produce Z11 positional isomers for pentacosene, but not for heptacosene, see Table 3.1. Assuming that this fatty acid is not modified in any way prior to use suggests that the resulting isomer must be a Z11, a position not normally produced by this species. The main positional isomers for heptacosene for *Myrmica sabuleti* are Z9 and Z13, these both co-elute in the main pentacosene peak and therefore if a Z11 was being biosynthesised then this would also co-elute with these other isomers. This means that if a Z11 compound were being synthesised then its incorporation would be detectable within the main pentacosene peak. It is worth stating that for heptacosene only one measurement was made for this compound. However this theory of co-elution would explain why there was no additional peak observed for this compound.

If this statistical significance is caused by incorporation of this fatty acid and therefore a Z11 isomer is being produced, this effect is only very subtle and certainly not similar to that seen for octadec-11-enoic acid-11,12-d_2. However as previously mentioned, again this is not a positional isomer normally produced by *Myrmica sabuleti*, but it is found in *M. scabrinodis* in moderate amounts. This suggests that it may therefore be another example of a biosynthetic pathway which is normally dormant, but can be

activated by the presence and usage of a suitable precursor.

This fatty acid substrate is one of the longest to be tested at twenty carbons in length. Biologically this is the most efficient fatty acid to use as a precursor as it requires the least amount of lengthening. This extra efficiency may explain why incorporation is only suggested for heptacosene and not seen for pentacosene as well, as the shorter molecule may be biosynthesised using a shorter precursor. Given that clear incorporation is not seen for this substrate it may be that chain length of the precursor is not a driving force behind usage.

6.3.12 Eicos-11-enoic acid-11,12-d_2

This was the final acid to be tested and it is commonly known as gondoic acid. It is found in various fish oils and is a constituent of rape oil in its glyceride form [12]. The number of samples for this substrate was 51. The following data refers to that obtained for the labelled substrate Eicos-11-enoic acid-11,12-d_2. Please see Figure 6.28 for the boxplots.

Figure 6.28 shows that the median percentage abundances for both groups are very similar, for both $I^{(M+1)}$ and $I^{(M+2)}$. It can however be seen that generally the range of the data is greater for the substrate group compared to the control group, for both measured levels. There are also far more outliers within the substrate group, which suggests that there may be some individual incorporation, rather than a group effect. In order to analyse statistical significance between the groups for all the compounds Mann-Whitney U-tests were performed. See Table 6.12.

The results of the U-tests indicate that tricosane is the only compound which is statistically significant at either level. This has a p value of 0.041 which is only just lower than the critical p value of 0.050. This suggests that any effect seen for this compound

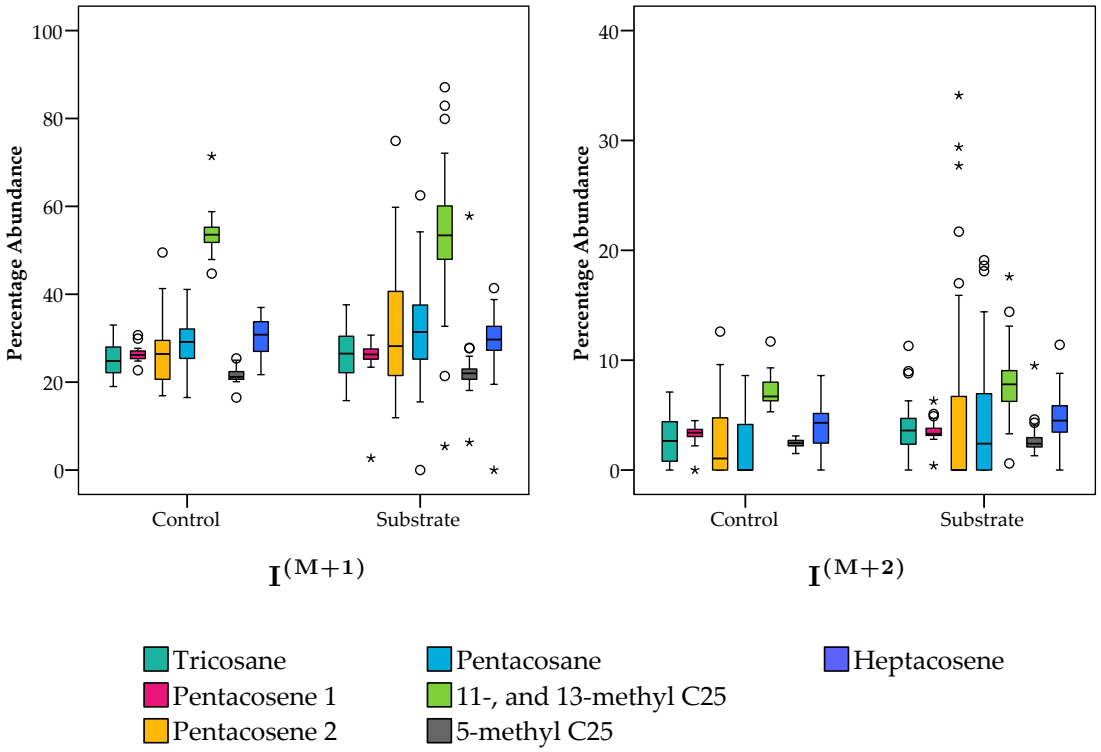


Figure 6.28: Boxplots showing the data for Eicos-11-enoic acid-11,12-d_2. The data for $I^{(M+1)}$ is on the left and $I^{(M+2)}$ on the right.

is only minor and therefore may be as a result of metabolic breakdown, as previously mentioned.

Looking at Figure 6.28, it can be seen that for $I^{(M+2)}$ there are a lot of outliers for the secondary pentacosene isomer. Looking at the raw data values shows that there are several samples which may be showing a very localised incorporation of the labelled substrate. For example the average control value for this compound is 3.1%, however for the substrate set of data there are four samples with comparative values of greater than 21%. All of these samples also have higher than average values for $I^{(M+1)}$ indicating that these are individuals which may be showing incorporation. See Appendices D.26-D.27. Unfortunately because there are so many samples not showing this same incorporation there is no overall statistical difference at group level.

This substrate is an ω-9 fatty acid, however as previously mentioned *M. sabuleti* only produce small amounts of Z9 pentacosene, the major isomer being the Z11 compound.

Table 6.12: The probability values from the calculated Mann-Whitney U-tests for Eicos-11-enoic acid-11,12-d_2. Note that any p values less than 0.050, and therefore significant, are underlined for ease of reference.

Compound	p value	
	Eicos-11-enoic acid-11,12-d_2	
	$I^{(M+1)}$	$I^{(M+2)}$
Tricosane	0.209	<u>0.041</u>
Pentacosene-1	0.408	0.463
Pentacosene-2	0.129	0.350
Pentacosane	0.116	0.123
11-, and 13-methyl C25	0.441	0.140
5-methyl C25	0.230	0.443
Heptacosene	0.404	0.127

Therefore any incorporation of this substrate would likely be only a minor process as the demand for the biosynthesis of this compound would be low. It is also worth considering that the most abundant fatty acid, oleic acid, is an ω-9 fatty acid, and so it is likely that there would be plenty of this compound available from the other components of the diet. This would mean that less of the labelled substrate would be required and thus less incorporation would be seen.

6.3.13 Discussion

This experiment tested the effects of twelve different substrates on the cuticular hydrocarbon profile of *Myrmica sabuleti*. The clearest and therefore most interesting results obtained from this experiment were those from octadec-11-enoic acid-11,12-d_2. The effects of this substrate revealed that when fed a diet rich in this compound *Myrmica sabuleti* ants started to produce a positional isomer that was not normally found within their profile. This Z7 pentacosene isomer is however found in the cuticular hydrocarbon profile of the closely related species *Myrmica scabrinodis*. Therefore one theory for this may be that both species share the same biosynthetic pathways, but only certain

pathways are active within each species, possibly in order to keep the species, which are found in similar locations, chemically different, in order to act as species specific cues. It is already well established that positional isomers in alkenes can act as both species specific and nestmate specific cues in the insect world. [16, 21, 22]. Whilst there is no additional supporting evidence for this theory it certainly does explain why a previously unseen positional isomer can be biosynthesised by this species.

Although a very clear result was obtained for the octadec-11-enoic acid-11,12-d_2 substrate, it is unknown why this was observed. In terms of the structure of this substrate it is a C18:1 fatty acid, i.e. the carbon chain is 18 carbons long and contains one double bond. However this is not a fatty acid which is commonly encountered in nature so it is unknown why this substrate yielded such a clear result when there are other substrates such as hexadec-9-enoic acid-9,10-d_2 which are much more common. If the incorporation was related to the natural abundance then it would be expected that the hexadec-9-enoic acid-9,10-d_2 would show the clearest incorporation, as this fatty acid would naturally make up part of the ants normal diet.

Throughout these experiments there were a variety of different results seen. Whilst there were very few trends across all the substrates tested, there were a few that showed incorporation at the $I^{(M+2)}$ for the compound pentacosane. There are two main theories for this trend. Firstly it could be that a secondary mechanism exists which involves the hydrogenation of an unsaturated fatty acid into the equivalent saturated fatty acid. This saturated fatty acid could then be converted into an alkane, and the deuterium labels retained. This process could be driven by the levels of precursors available and in a situation where there are less saturated fatty acids available this mechanism may become more important. However it is worth considering that within this species pentacosane is not a major compound of the chemical profile, so it is unlikely that there would be a high demand for saturated fatty acids.

The other theory as to why this trend is observed relates to the control values. Unusually for this experiment the controls were not run alongside the substrates. This would have meant twice the synthetic work as each substrate fatty acid synthesised would have to be produced in both the labelled and unlabelled version. Instead for this experiment the control samples were taken from a selection of previously obtained *Myrmica sabuleti* extracts. As a consequence of this, the same twenty control extracts were used for each substrate experiment. The significance seen for pentacosane for so many of the substrates may be due to the comparative control value used. According to Equation 3.3 in Section 3.4.2.1 the theoretical value for $I^{(M+2)}/I^{M}$ can be calculated for pentacosane at 3.75%. The actual control value obtained from the real extracts is considerably lower at 2.2%. This may be introducing a false statistical significance into the substrate results. Interestingly when the calculated control value of 3.75% is used to calculate statistical significance i.e. twenty values of 3.75% then there is no statistical significance shown. However this reflects the nature of using genuine samples; they do not always reflect the ideal.

For some of the samples there is incorporation at the $I^{(M+1)}$ level only. This is unexpected as incorporation of the labelled fatty acid should yield an alkene with an extra two mass units, and thus a significant result for $I^{(M+2)}$. Whilst there may be some significance seen for $I^{(M+1)}$ due to hydrogen exchange, this is a minor process and so the difference observed at this level is likely to be much smaller than that observed for $I^{(M+2)}$. Therefore if there is incorporation at the $I^{(M+1)}$ level only, then there must be some mechanistic process that causes this. The limited results which show 1 Da of incorporation could be due to the metabolism of this compound and its subsequent breakdown into smaller constituents which are subsequently used during the biosynthesis of hydrocarbons. One of the main routes for the metabolism of fatty acids is β-oxidation. This metabolism is driven by the need for energy as well as a breakdown pathway. During this mechanism the length of the carbon chain within the fatty acid is gradually broken down, releasing a molecule of acetyl-CoA as carbons are used up

in the formation of the acetyl group [1]. This process primarily uses saturated acids such as stearic acid, however it can also utilise unsaturated fatty acids. In this case the eventual breakdown of the fatty acid via this mechanism would result in a single acetyl-CoA with a deuterium label attached. The other deuterium would be lost as part of the β-oxidation cycle [1]. The schemes shown in Figures 6.29 and 6.30 show this process.

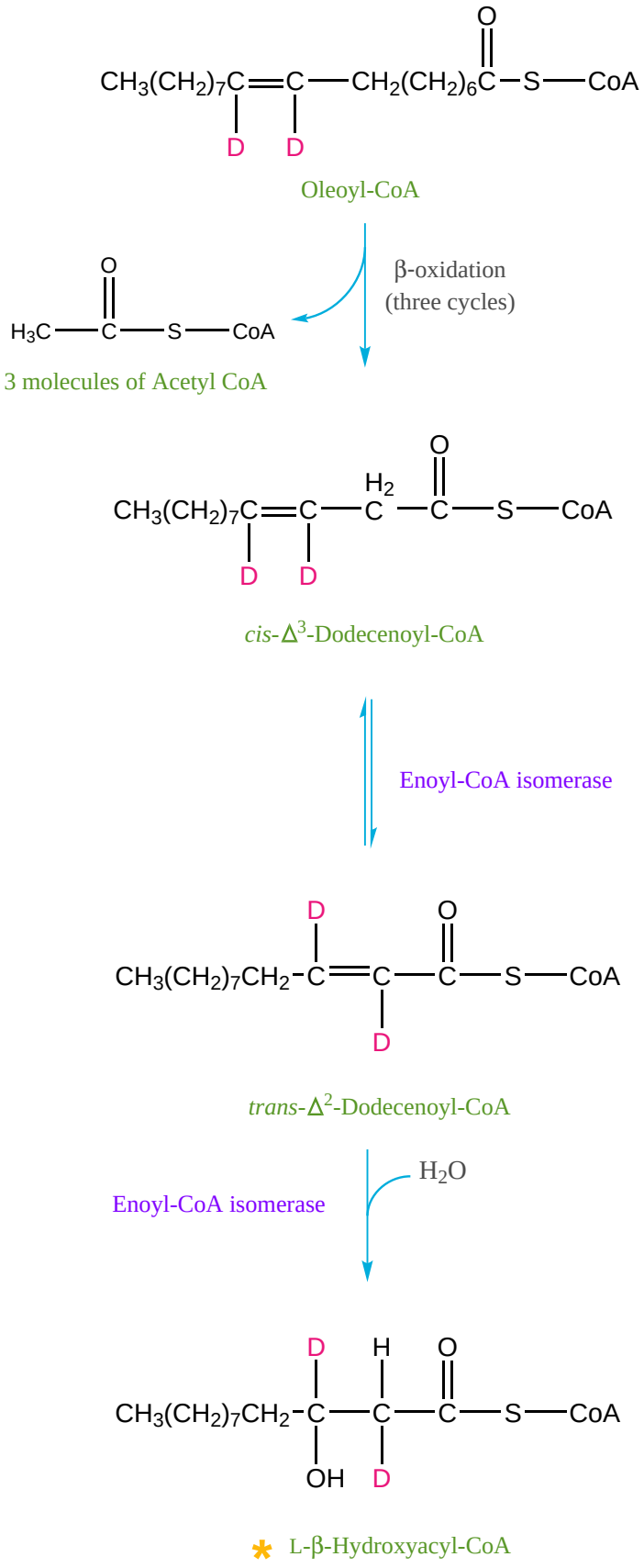


Figure 6.29: The reaction scheme above shows the initial steps in the β-oxidation of octadec-11-enoic acid-11,12-d_2. The product of this reaction can then continue within the β-oxidation cycle.Adapted from [1].

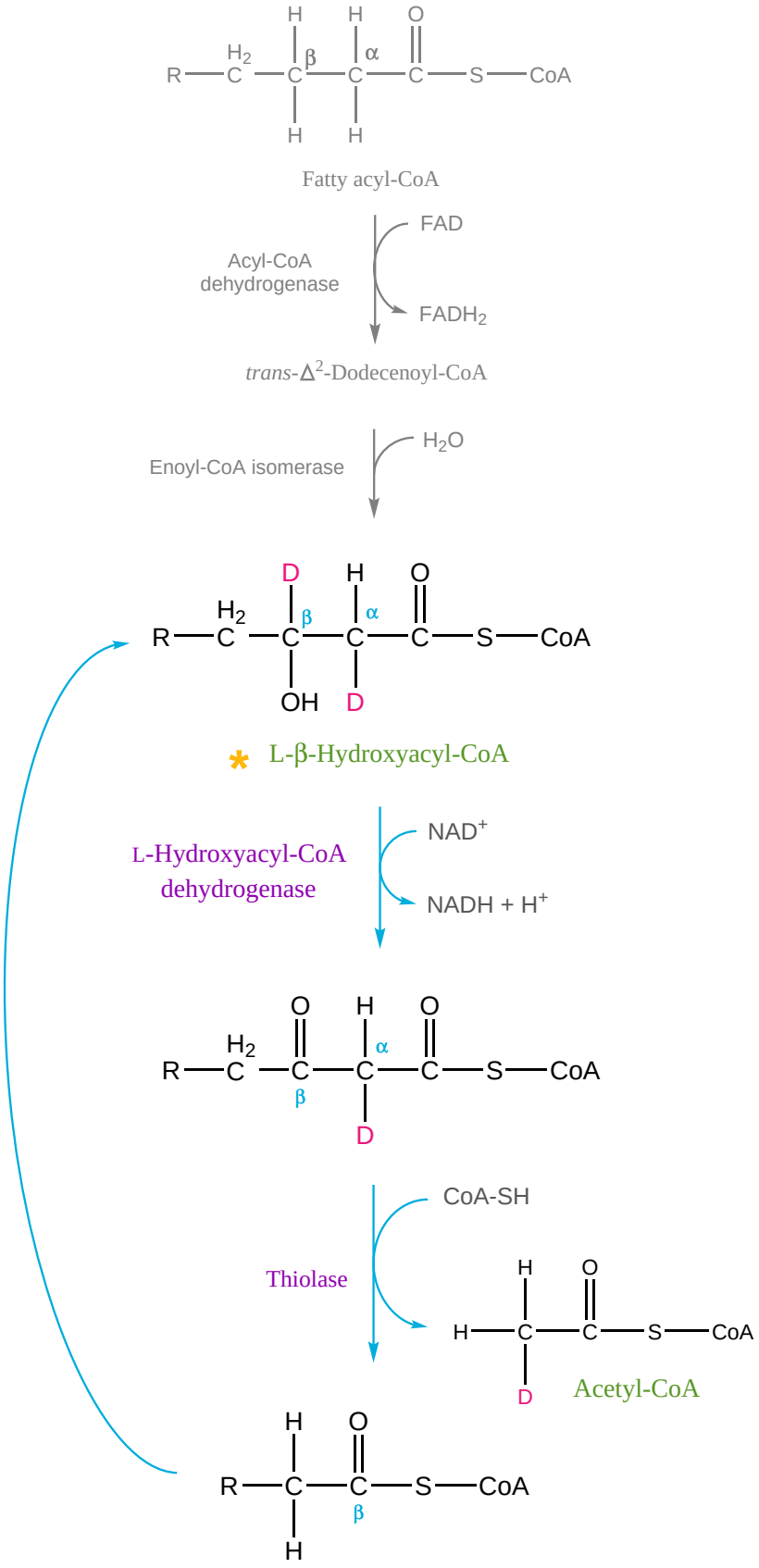


Figure 6.30: The reaction scheme above shows the final steps in the β-oxidation of octadec-11-enoic acid-11,12-d_2. This reaction produces an acetyl-CoA molecule with one deuterium present. Note that the greyed out part of this mechanism refers to the breakdown of a saturated fatty acid and is not relevant to this discussion. Adapted from [1].

Figure 6.29 shows that the initial steps of the breakdown are the removal of the chain up to the double bond, this occurs via the standard β-oxidation cycle. Three repetitions of this cycle reduce the carbon chain to 12 carbons long, with the labelled *cis* double bond now between carbons three and four. At this point a slightly different reaction occurs because double bond is in the *cis* configuration and for hydratase of the β-oxidation cycle it needs to be in the *trans* configuration. During this step a *cis-trans* isomerase acts upon the molecule and replaces the *cis* double bond between carbons three and four with a *trans* double bond between carbons 2 and 3. At this point the conventional β-oxidation cycle can resume as shown in Figure 6.30. Note that the initial steps of this mechanism are shown in grey as they depict the breakdown of a saturated fatty acid, a process not relevant to this discussion. The final product of this oxidation cycle feeds back into the reaction to undergo subsequent breakdown. The cycle repeats until the whole of the carbon chain is broken down into acetyl units. As these figures show for every molecule of labelled fatty acid broken down in this fashion, one molecule of acetyl-CoA is produced with a single deuterium atom present.

Acetyl molecules are extremely useful for biosynthesis as they can be used as part of the chain lengthening process to produce alkanes, alkenes or methyl-branched hydrocarbons. As the mechanisms contained within Sections 1.2.2.2 and 1.2.2.3 indicate, the production of an acetyl molecule with a single deuterium present would result in a long chain hydrocarbon being produced with this label still intact. This would lead to a detected increase in the intensity of the $I^{(M+1)}$ peak and hence the percentage abundance of $I^{(M+1)}/I^{M}$. As the results of these substrates showed, there are many occasions whereby there is a detectable mass increase of one unit, but not two. As shown, this could be caused by the breakdown of a labelled fatty acid into more biologically favourable molecules. This may also explain why on occasion a methyl-branched hydrocarbon also shows this mass increase. However because these labels are deuterium based no further study can be done on this incorporation to confirm its presence and determine its location.

One of the main issues surrounding these experiments is the lack of control over variables. As far as is possible the variables within the experiments are controlled. However it is impossible to control the variables surrounding the ants themselves. As previously discussed these ants are wild caught; fragments are collected from the wild and transferred to holding tanks, see Figure 3.1. They are kept within these tanks until they are required and, during this time, they are fed a standard diet [23]. This diet has an agar jelly base, and to this egg whites, honey, and vitamins and minerals are mixed. In theory this should provide all the nutrients that the ants require, however it cannot possibly replicate that which the ants would consume from varied foraging. Therefore during the time that these ants are kept in the lab their natural stores will become depleted, this may have an effect on the amount of incorporation seen. This is particularly relevant to the incorporation of fatty acids. As well as being a starting material for the synthesis of hydrocarbons, fatty acids are also used as energy stores in the form of triacylglycerols [5]. These are stored in the adipocytes; these are the main fat body cells [1, 24]. When extra energy is required these fatty acids are metabolised to form acetyl-CoA, as shown in Figures 6.29 and 6.30. The acetyl-CoA molecules are then converted to form adenosine triphosphate (ATP) via the citric acid cycle [1]. Therefore it is possible that ants which have been kept in an artificial environment for a long time are starved of natural resources and possibly energy. If this is the case, they may become depleted in fat stores and thus levels of fatty acids may be low within the ants' systems. Therefore any fatty acids introduced via diet would immediately be used for possibly energy and/or hydrocarbon biosynthesis. This means that, although not investigated, there may be some link between the 'lab age' of the ants and their readiness to incorporate a substrate.

The diet that the ants are fed during the substrate experiment is based on that by Bhatkar and Whitcomb [23]. However it has been modified; the original diet used one whole egg, this has been substituted within this project for powdered egg white. This exchange was chosen to minimise waste as one batch of the diet outlined in Bhatkar and

Whitcomb produced over half a litre of liquid diet [23]. Instead, using egg white meant that very small batches could be made without causing excess waste and consumption of materials. Although not ideal the exchange of raw egg for egg white ensured that the protein content was concentrated. However it also meant that the nutrients found in egg yolks were not included within this diet. The composition of a dry egg yolk is mostly lipids (62.5%), followed by proteins (33.0%). The other minor components of hen egg yolk are minerals and carbohydrates [25]. Specifically within this lipid component there are many fatty acids present, however the majority of this lipid component (86%) is formed of oleic, palmitic and linoleic acids [26]. The egg white or albumen contains only trace amounts of fats, instead dried egg white is mostly protein [27]. According to the same source there is no also fat present in honey [27]. Given that these ants do not consume high amounts of fats it is logical to assume that over time these stores would become depleted. Therefore it is possible that the natural stores of fatty acids is quite an important factor in this experiment and may explain why varied levels of incorporation is seen.

As previously mentioned the substrate experiments involving fatty acids were run in three blocks, the first of which tested octadec-11-enoic acid-11,12-d_2. The ants that were used for this experiment were taken from the wild some months earlier, from the previous autumn. Therefore the ants used for this experiment may have been effectively starved of natural resources and precursors, this may explain why significant incorporation was seen for this substrate and not for others. The other substrates required a very large number of ants and as such these were collected specifically for this experiment at the start of the season. They were kept for a very short time before being used for the experiment. Hence they would have had an abundance of natural stores including larger amounts of lipids and fatty acids. This may mean that newly collected ants will show very little incorporation of any compound, simply because they do not need it, as natural foraging has equipped them with high reserve levels of required fatty acids and other biosynthetically favourable building blocks. Therefore,

although not investigated, this again suggests that there may be some link between the 'lab age' of the ants and their incorporation of a substrate.

6.4 Conclusions

This experiment showed some interesting results. The first, and arguably the most significant, was the result that indicated that, when fed a diet containing 3% octadec-11-enoic acid-11,12-d_2, *Myrmica sabuleti* produce a Z7 positional isomer of pentacosene, which is not normally a component of their chemical profile. This is a significant result as it may suggest that this species contains deactivated biosynthetic routes which can be reactivated if required. The closely related species *Myrmica scabrinodis* does produce this isomer and therefore there may be some degree of shared pathways between the two species. This suggests that there may be flexibility surrounding the compounds that can and cannot be produced by certain species.

There were also several substrates that produced results which were not as conclusive as those seen for octadec-11-enoic acid-11,12-d_2. The reasons for these observations have been largely explored within the relevant sections, however it is worth considering that, as with all biologically important molecules, they can be readily metabolised to form smaller 'building block' type molecules. These in turn could be incorporated into a variety of different molecules at varying stages leading to the irregularity of the results. Despite the irregularity of these results, and the consequent lack of clear trends, further analysis of the results in terms of the raw data, suggest that the observations are not always due to anomalous data or instrumentation issues. As such, supporting theories for the various findings of this chapter are postulated; it is clear that there is plenty of scope for additional research within this area.

References

[1] Campbell MK, and Farrell SO, *Biochemistry 8th Edition*, (Cengage Learning, 2015)

[2] Morgan ED, *Biosynthesis in Insects*, (Royal Society of Chemistry, 2010)

[3] Blomquist GJ, *Insect Hydrocarbons*, (Cambridge University Press, 2010)

[4] Howard RW, and Blomquist GJ, 'Ecological, behavioural, and biochemical aspects of insect hydrocarbons,' (2005), *Annu. Rev. Entomol.*, 50, 371-393

[5] Stanley-Samuelson DW, Jurenka RA, Cripps C, Blomquist GJ and De Renobales M, 'Fatty acids in insects: Composition, metabolism and biological significance,' (1988), *Arch. Insect Biochem. Physiol.*, 9, 1-33

[6] Carlson DA, Roan CS, Yost RA, and Hector J, 'Dimethyl disulfide derivatives of long chain alkenes, alkadienes and alkatrienes for gas chromatography/mass spectrometry,' (1989), *Anal. Chem.*, 61(14), 1564-1571

[7] Spencer GF, Kleiman R, Miller RW, and Earle FR, 'Occurrence of cis-6-hexadecenoic acid as the major component of *Thunbergia alata* seed oil,' (1971) *Lipids*, 6(10), 712-714

[8] Agilent Technologies, *5975 Series MSD Troubleshooting and Maintenance Manual, 4th Edition*, (Agilent Technologies, 2012)

[9] Martin SJ, Shemilt S, and Trontti K, 'Nest-mate recognition cues are not used during or influenced by mating in the ant *Formica exsecta*,' (2013), *Ethol. Ecol. Evol.*, 26, 40-48

[10] Wagner D, Tissot M, and Gordon DM, 'Task-related environment alters the cuticular hydrocarbon composition of harvester ants,' (2001), *J. Chem. Ecol.*, 27, 1805-1819

[11] Frentiu FD, and Chenoweth SF, 'Clines in cuticular hydrocarbons in two *Drosophila* species with independent population histories,' (2010), *Evolution*, 64-66, 1784-1794

[12] Blunt JW, and Munro MHG, *Dictionary of Marine Natural Products*, (CRC Press, 2007)

[13] Degirolamo C, and Rudel LL, 'Dietary monounsaturated fatty acids appear not to provide cardioprotection,' (2010), *Curr Altheroscler Rep.*, 12(6), 391-396

[14] British Wasp and Ant Recording Society; *Myrmica sabuleti* reference page http://www.bwars.com/ant/formicidae/myrmicinae/myrmica-sabuleti (accessed July 2017)

[15] Sudd JH, and Franks NR, *The Behavioural Ecology of Ants*, (Blackie, 1987)

[16] Guillem RM, Drijfhout FP, and Martin SJ, 'Using chemo-taxonomy of host ants to help conserve the large blue butterfly,' (2012), *Biol. Conserv.*, 148(1), 39-43

[17] Fatima T, Snyder CL, Schroeder WR, Cram D, Datla R, Wishart D, Weselake R, and Krishna P, 'Fatty acid composition of developing Sea Buckthorn (*Hippophae rhamnoides* L.) berry and transcriptome of the mature seed,' (2012), *Plos One*, 7(4), 1-18

[18] Pech P, and Prusková K, *Myrmica scabrinodis* as a possible host of *Myrmica hirsuta* (Hymenoptera: Formicidae),' (2013), *Entomol. Fennica*, 24, 140-141

[19] Jansen G, Savolainen R, and Vepsäläinen K, 'Phylogeny, divergence-time estimation, biogeography and social parasite–host relationships of the Holarctic ant genus *Myrmica* (Hymenoptera: Formicidae),' (2010), *Molecular Phylogenetics and Evolution*, 56, 294-304

[20] Guillem RM, Drijfhout FP, and Martin SJ, 'Species-specific cuticular hydrocarbon stability within European *Myrmica* ants,' (2016), *J. Chem. Ecol.*, 42, 1052-1062

[21] Martin SJ, Carruthers JM, Williams PH, and Drijfhout FP, 'Host specific social parasites (*Psithyrus*) indicate chemical recognition system in bumblebees,' (2010), *J. Chem. Ecol.*, 36(8), 855-863

[22] Nascimento DL, and Nascimento FS, 'Acceptance threshold hypothesis is supported by chemical similarity of cuticular hydrocarbons in a stingless bee, *Melipona asilvai*,' (2012), *J. Chem. Ecol.*, 38, 1432-1440

[23] Bhatkar A, and Whitcomb WH, 'Artifical diet for rearing various species of ants,' (1970), *Florida Entomol.*, 53(4), 229-232

[24] Arrese EL, and Soulages JL, 'Insect fat body: Energy, metabolism, and regulation,' (2010), *Annu. Rev. Entomol.*, 55, 207-225

[25] Anton MM, *Composition and Structure of Hen Egg Yolk*, In 'Bioactive Egg Compounds' (Huopalahti R, López-Fandiño R, Anton M and Schade R) (Springer Berlin Heidelberg, 2007)

[26] Bitman J, *Status Report on the Alteration of Fatty Acids and Sterol Composition in Lipids in Meat, Milk and Eggs*, In 'Fat Content and Composition of Animal Products: Proceedings of a Symposium' (National Research Council) (National Academy of Sciences, 1976)

[27] Food Standards Agency, *McCance and Widdowson's The Composition of Foods 6th summary Edition.*, (The Royal Society of Chemistry, 2002)

Chapter 7

A study into the relationship between substrate incorporation and amount of food consumed

7.1 Introduction

7.1.1 Background

The work presented so far within this thesis has demonstrated that there are some interesting results shown when ants are fed a labelled substrate. Such substrates are generally biologically important molecules such as amino or fatty acids, or acetates and propionates, and are labelled with stable isotopes such as ^{13}Carbon or deuterium atoms. The amount of incorporation and the compounds into which these isotopic labels are incorporated appear to have some dependence on the compound type; alkene, alkane or methyl-branched hydrocarbon, and the experimental species. However undoubtedly the main influence on the degree of incorporation is most likely the amount of food eaten. Whilst there are probably many additional unknown factors, incorporation will not be possible unless the food is consumed. These unknown factors mean however that it does not necessarily follow that greater labelled diet consumption will lead to greater incorporation. The diet into which the labelled substrates are added to is based on a very well established diet by Bhatkar and Whitcomb [1]. This diet is based on a simple agar jelly matrix into which the substrate compound can be added at a w/w concentration of generally 1-3%. However due to the jelly matrix this diet is very prone to water loss, this leads to a decrease in the mass of the food. In the

same way, water absorption would lead to a resulting mass increase. This means that being able to track the amount of food consumed over the course of an experiment is impossible via conventional methods; i.e. monitoring changes in mass of the food. The change in food mass due to the loss or gain of water will far outweigh the very small mass change due to consumption. It is therefore very difficult to understand exactly how much food is consumed over the course of an experiment.

It is also extremely likely that whilst ants demonstrate trophallaxis, or mutual feeding, [2, 3] some ants will consume a larger amount of food than others. This difference might be due to age, status or health, and again global changes in the mass of the diet will not show any indication as to an individual's feeding habits. Previous experiments have featured an additional labelled substrate diet, normally containing 2% w/w sodium [1-^{13}C]acetate. This was to ascertain whether a negative result within an experiment could be due to low amounts of feeding occurring. From previous work, see Chapter 4, sodium [1-^{13}C]acetate was known to provide a reliable positive result. Therefore by feeding this substrate alongside an untested substrate the possibility of a negative result from under-feeding, or even lack of feeding could be removed, assuming that a positive result was seen with the sodium [1-^{13}C]acetate substrate. However even this basic measure assumed that the feeding response would be the same between populations, which it might not have been, again due to inherent differences between groups of ants, or even the possibility that some diets may simply taste better or be more attractive than others. Therefore the aim of this final chapter was to devise a way of comparing the amount of food consumed and the incorporation seen and determining whether a link could be found between the two.

Previous studies by other researchers have removed this problem by injecting the substrates directly into the haemolymph of the study species [4–7]. This has the benefit that a known amount of substrate can be introduced consistently, however this method is not suitable for our study species, as their small sizes make injection impossible

without inflicting lethal amounts of trauma. Therefore due to their small sizes it was necessary to introduce the labelled substrates via feeding experiments. However as mentioned above this means that there is no way of knowing through standard feeding experiments how much food is consumed and how this relates to the amount of incorporation seen. Therefore for the final few experiments the experimental diets were again modified to include a fluorescent dye. It was hoped that this fluorescent dye would be consumed and would then effectively stain the haemolymph of the ant in a cumulative fashion. In this way the amount of incorporation and the amount of dye could be measured to see if there was any apparent link between the two.

7.1.2 Fluorescence

7.1.2.1 Mechanism of fluorescence

Fluorescence is a form of rapid radiative decay and is defined as the subsequent re-emission of characteristic wavelengths of light following the absorption of radiation by a material [8, 9]. In accordance with Stokes' law the wavelength of light corresponding to the maximum intensity of re-emission will always be a longer wavelength than that of the original excitation [10]. Although the full detail of the underlying mechanism behind fluorescence is beyond the scope of this thesis the basic mechanism underpinning it can be presented using a simplified energy diagram.

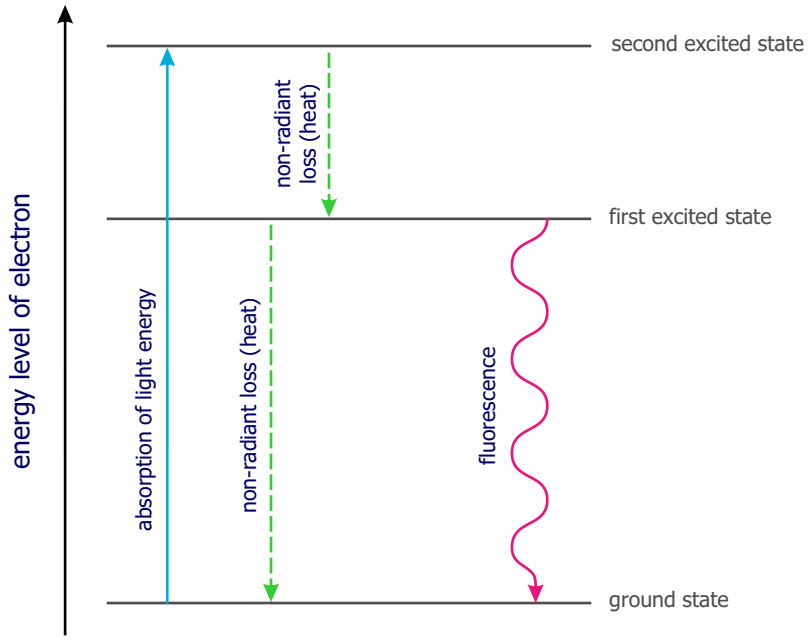


Figure 7.1: Basic energy level diagram representing the mechanism of fluorescence based on [11]. Upon absorption of specific wavelengths of electromagnetic radiation, electrons are promoted from the stable ground state to unstable higher energy levels. Some of this absorbed energy is lost via non-radiant routes and the rest is emitted as electromagnetic radiation [11].

Fluorescence is most often associated with rigid cyclic molecules which possess π-bonds. Such bonds are found in high numbers in aromatic compounds, meaning that highly coloured compounds, including those which fluoresce, are often made up of a highly conjugated ring system. The associated fluorescence with these molecules is further enhanced by the presence of electron donating groups i.e. substituents with lone pairs of electrons. Conversely the fluorescent effects are reduced by electron withdrawing groups [9, 10]. Highly fluorescent dyes also generally possess a highly rigid structure. This high degree of rigidity means that energy loss due to intramolecular motion is minimised, therefore fluorescence is favoured over non-radiative energy loss [12].

7.1.2.2 Fluorescent dyes

Fluorescent materials are widely used in many areas of chemistry, biology and forensic science [13–18]. The review of insect marking by Hagler found that the addition of various dyes to food has been used within entomological studies since the 1960s and that selected dyes must be adequately retained by the insect, easily detectable and non-lethal, even in high doses [19]. More recently fluorescent materials have been used in studies by many researchers for a variety of purposes [20–22]. Tschinkel used several different colours of fluorescent dye to spray workers in order to readily mark, and subsequently detect different castes. However like the other studies mentioned above, this study also used the fluorescent dye rhodamine B to stain food. As well as the study by Tschinkel, rhodamine B was also used in the study by Gayahan to stain small pieces of the tenebrionid beetle larvae *Zophobas atratus* [21,22]. In the study by Greenwald, rhodamine B was added to liquid food solutions of sucrose, glucose and Bovine Serum Albumen (BSA) in order to measure the food load of all the individuals within a colony [20]. All of these studies found that the use of fluorescent dyes was successful and allowed for workers to be internally stained by feeding them dyed foodstuff. In particular the study by Greenwald found that the presence of the rhodamine B dye was detectable within the crop of the ant through the thickness of the gaster and thus allowed for the movement of liquid nutrients to be detectable through workers. This study also found that rhodamine B was suitably water soluble and non-lethal to the ants, even in doses up to 10 g/L [20].

Rhodamine B

As previously discussed rhodamine B is a highly fluorescent commercially available dye with a variety of uses [23–26]. It is a member of the Rhodamine family of dyes and is classed as a xanthene type dye. Xanthenes are characterised by the xanthene core which consists of a system of 3 cyclic rings at the centre of the molecule [27]. The inclusion of an oxygen bridge within the chemical structure lends this molecule increased rigidity and as a result increased fluorescence [12]. As previously discussed rhodamine

B has a variety of uses and has a solubility of 1 mg/mL in water and an optimum excitation wavelength of 553 nm and an emission of 627 nm [28]. The structure of rhodamine B is shown in Figure 7.2.

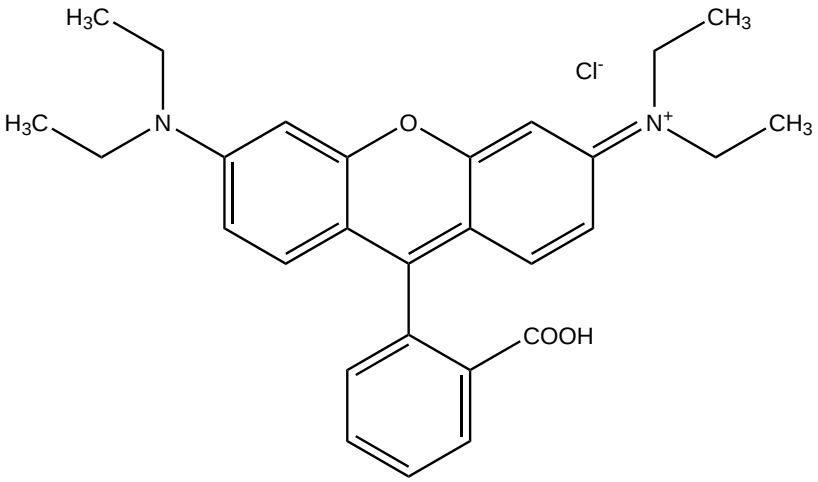
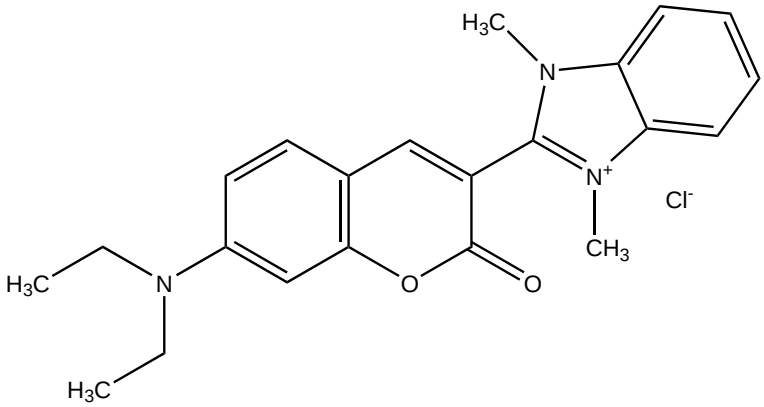


Figure 7.2: The structure of the fluorescent dye rhodamine B, one of two used in this experiment [28]

Basic yellow 40

The fluorescent dye basic yellow 40 belongs to the coumarin family of dyes. Fluorescent coumarins absorb and emit in most parts of the visible spectrum although most are characterised by a greenish-yellow fluorescence [12]. Although used in the textile industry, basic yellow 40, known from here on in as basic yellow, is most commonly used in forensic science as a dyeing agent during a process used to enhance latent fingerprints on plastics [29]. The general excitation is accepted to be around 450 nm and the emission around 495 nm [30]. The structure of basic yellow can be seen in Figure 7.3.



Basic Yellow 40

Figure 7.3: The structure of the fluorescent dye basic yellow, one of two used in this experiment [30]

7.1.2.3 Fluorescence spectrophotometry

There are many fluorescent dyes commercially available and as such they have known optimum excitation and emission wavelengths. For example the dye Fluorescein has an optimum excitation wavelength of 490 nm and an emission of 514 nm, [28] with the difference between excitation and emission wavelengths defined as the Stokes shift, typically 50-70 nm for fluorescent dyes [11, 12]. The specialised emissions of these fluorescent dyes mean that there are several methods that can be used to measure the amount of resulting visible light.

One of the most well-known instrumental techniques used to measure fluorescence is fluorescence spectrometry, also known as fluorimetry. Generally a bench top fluorimeter contains two grating monochromators, each one capable of scanning a spectral band. During use one monochromator selects the emission band whilst the other scans the excitation band, alternatively the excitation band is fixed whilst the emission band is scanned. If the optimum emission and excitation wavelengths are not known then the fluorimeter can be used to detect them. However in the case of commercial dyes these parameters are known and therefore the fluorimeter can be used to accurately analyse the amount of relative fluorescence from a sample [11]. A similar technique

to the fluorimeter is the fluorescence microplate reader. This instrument works in a similar way to the fluorimeter however it is designed to measure fluorescence on a much smaller scale, and it is often used within biological applications. Microplate readers allow the amount of fluorescence present within each sample to be quickly measured. Each microplate contains a number of wells which the samples can be loaded into, the number of wells typically varies with 96 wells being the most common. The size of the well can vary too, with some plates featuring wells as small as 50 μL [31].

For the purposes of measuring the fluorescence within this project a fluorescence microplate reader was selected as being the best technique. Using this highly sensitive technique would allow the fluorescence of each of the small volumes of sample to be measured. However due to Covid-19 restrictions and thus circumstances beyond control, this technique could no longer be used. Instead a system utilising digital photography was designed to measure the resulting fluorescence of each sample. Fluorescence photography is a technique which is commonly used within the field of forensic analysis for a variety of purposes [11]. The principles of this technique are the same; an intensely coloured light source, designed for fluorescence imaging, provides an excitation source for each sample. A high-pass filter placed in front of the digital camera ensures only the weakly fluorescent light emitted by the sample is gathered [11]. Once the images are taken the amount of fluorescence can be compared between samples and the amounts of relative fluorescence determined.

Aims & Objectives

The primary aim of this chapter is to test the theory that the amount of incorporation of the stable isotopes, present in the modified diets, is directly related to the amount of food consumed. The previous theories presented in this work are based upon the fact that it is currently unknown whether there is a direct relationship between incorporation and amount of food consumed or whether there is an additional key factor, such as pre-existing internal stores of biologically important molecules. In this manner an

ant may consume a high proportion of food yet not show similarly high incorporation due to the fact that this ant already has large stores of molecules which can be used for biosynthesis. By determining whether there is a direct link between the two variables, stronger conclusions can be derived from the previously presented chapters.

7.2 Experimental

7.2.1 Experimental set-up

The aim of this experiment was to compare the amount of food consumed and the incorporation seen in the biosynthesised hydrocarbons and thus determine whether there is a direct link between the two. As such it was planned that this experiment would involve the three ant species used predominantly in this thesis, namely; *Formica lemani*, *Myrmica sabuleti* and *Myrmica scabrinodis*. However as previously mentioned the use of these species in this research relies on fragments of wild colonies being collected and thus being readily available. Despite best efforts, due to limitations on time, the collection of either *Myrmica sabuleti* or *Myrmica scabrinodis* in sufficient numbers was not possible. For this reason the use of these two species was necessarily substituted with that of *Myrmica rubra*, a more widely available, though previously unused Myrmica ant. Supplies of this species were readily available from the same area of the Peak District as that of *Myrmica sabuleti* and *Myrmica scabrinodis*. In addition it was observed, after chemotaxonomic identification, that the colony fragments collected which were all initially assumed to be *Formica lemani* were in fact a mixture of *Formica lemani* and the morphological similar species *Formica fusca*. Therefore this experiment was carried out with three species: *Formica lemani*, *Formica fusca* and *Myrmica rubra*.

In addition two fluorescent dyes were selected for experimental use, this was in order to determine if there was any difference between results, due to either ant preference, toxicity or instrument sensitivity. The first dye selected was rhodamine B; chosen due to its widespread use in studies of this type, its highly fluorescent properties and its non-toxic nature, as previously described [20–22]. The second dye chosen was basic yellow, although its use in studies of this type has not been described, it was felt that inclusion of this dye would be useful as it would provide an extra experimental variable. Three concentrations of the dye were selected based on the toxicity tests of Greenwald mentioned above [20]. These concentrations were 1 g of dye per 1 litre of liquid food,

2 g/L and 5 g/L as it was not known what effect these dyes would have on these ants. For each concentration there were three repeats. Each population was housed in a circular container (diameter 12 cm and depth 8 cm) and the sides of each container were painted with Fluon (Blades Biological Ltd.) and a small amount of soil placed inside.

For this experiment nine separate diets were required. These were all based on the standard agar diet according to that described by Bhatkar and Whitcomb [1]. Seven of these nine diets contained 1% w/w of sodium [$^{13}C_2$]acetate (Sigma Aldrich), and to six of these seven different concentrations of basic yellow or rhodamine B were added. The final of these seven did not have any dye added to it and acted as a substrate only control. In addition to these seven diets, two additional diets were kept free of sodium [$^{13}C_2$]acetate, in order to act as dye-only controls; these diets having a dye concentration of 2 g/L. Table 7.1 shows a summary of the nine diets and their formulations.

Table 7.1: A table showing the composition of the nine diets used in the fluorescence experiment. Note that the sodium acetate referred to below was isotopically labelled as previously stated.

Dye	Concentration	Sodium acetate	Purpose
Rhodamine B	5 g/L	1%	Experimental
	2 g/L	1%	Experimental
	1 g/L	1%	Experimental
	2 g/L	No	Dye only control
Basic Yellow	5 g/L	1%	Experimental
	2 g/L	1%	Experimental
	1 g/L	1%	Experimental
	2 g/L	No	Dye only control
None	N/A	1%	Sodium acetate control

In order to cut down on the amount of samples, and due to species availability, the experiment was designed to test multiple variables as efficiently as possible. Therefore to test the possible differences due to the choice of dye *Myrmica rubra* was tested against

both of the chosen dyes. For the *Formica* species, of which there were limited numbers, each was allocated an experimental dye to be tested against, this would allow a third species to be investigated whilst ensuring that there were enough individuals from each species.

For each diet described above there were three repeats with the exception of the controls, of which there was only the one box. Figure 7.4 summarises the experiment.

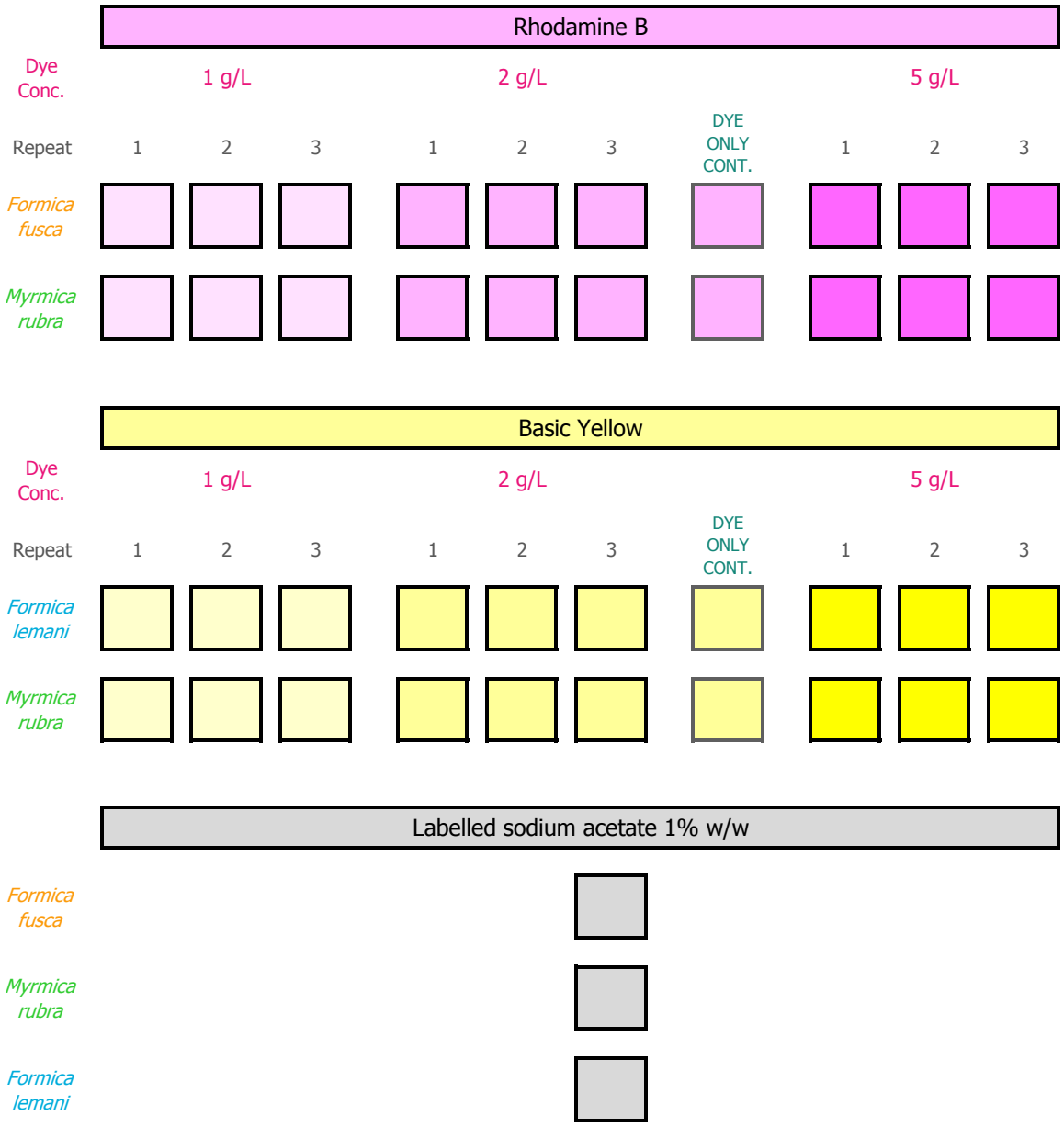


Figure 7.4: The experimental setup for the fluorescence experiment; each pink, yellow or grey box represents an experimental container. In total there were 43 experimental containers. Note that all boxes apart from those labelled as dye only controls, contained 1% w/w sodium [$^{13}C_2$]acetate.

All diets were cut into small cubes (approximately 0.5 cm^3). One cube was placed on to a small piece of plastic inside each box and the food was replaced every other day. All diets were stored in the freezer and defrosted prior to use each time. Originally it was planned to cease the whole experiment after 30 days, however mid-way through the experiment this plan was changed, as it was thought unfeasible due to time to stop

the whole experiment on the same day. Therefore extraction of *Formica lemani* and *Formica fusca* occurred on day 26. The extraction of *Myrmica rubra* was planned to be on day 28, however this was brought forward by 24 hours as rapid mortality was observed with this species in the end stages of the experiment. This was suspected to be due to excessive laboratory temperatures ($\sim$ 30 °C) due to a heat wave, therefore this experiment was stopped on day 27.

7.2.2 Sample analysis

7.2.2.1 Hydrocarbon analysis

Upon cessation of each part of the experiment all remaining ants from each of the 43 boxes were individually extracted in accordance to the methods described in Section 3.2.1. However there were a few changes made to this method. Following removal from the hexane after extraction, each ant was placed into a correspondingly labelled 1.5 mL Eppendorf tube and 0.5 mL of acetonitrile was added. This was so that each GC-MS profile could be related back to a stored ant sample ready for fluorometric analysis. All samples were stored at -25 °C prior to analysis. In addition to the changes described above, the GC-MS method used for analysis was also modified. This was to enable a shorter, more focussed run time due to the large volume of samples generated during this experiment. All chemical extracts were analysed using an Agilent 6890 gas chromatograph coupled to an Agilent 5973 MSD quadrupole mass spectrometer (MS -70 eV, electron impact ionization) with a quadrupole mass analyser. The GC was equipped with a HP–5MS column (length, 30 m; ID, 0.25 mm; film thickness, 0.25 μm; Agilent Inc. USA). The oven temperature programme was initially 80 °C and this was held for one minute. A temperature ramp of 35 °C min^{-1} to 210 °C was then performed followed by a slower temperature ramp of 5 °C min^{-1} to 280 °C. Finally there was a 40 °C min^{-1} temperature ramp to finish at 320 °C. This temperature was held for one minute. All samples were injected in splitless mode with the injection port heated to 325 °C and the MS in scan mode. Helium was used as the carrier gas and the flow

rate was 1.0 mL min^{-1}. All hydrocarbons were identified using diagnostic ions and Kovats indices [8]. All spectral analysis was performed using the Agilent ChemStation software.

As previously described in Section 3.4.2.1 the percentage abundances were recorded for each of the samples. However in order to ensure that the results of any incorporation were as accurate as possible only values for the two most abundant compounds were recorded. This was to try to prevent the previously observed anomaly whereby some less abundant compounds have very low ion counts and as such, can show highly inaccurate percentage abundance values. As the precision for the amount of incorporation for this study was so important it was therefore decided to focus only on the most abundant, and therefore the most accurate compounds. For both *Myrmica rubra* and *Formica fusca* these compounds were pentacosane and heptacosane, and for *Formica lemani* these compounds were pentacosene and heptacosene. Once the percentage abundances had been recorded for M+1 and M+2, the mean of these values was found and according to this mean, a rank was allocated; one representing the highest mean value.

7.2.2.2 Fluorescence analysis

Following the chemical analysis process all samples were returned to the freezer with each ant stored in 0.5 mL of acetonitrile. In order to the prepare the samples for fluorescence analysis each ant, contained within an individual Eppendorf tube, was crushed using the narrow end of a 10 mL pipette tube. This was in order to release the fluorescent dye contained within the tissues of the ant's body. Once crushed the Eppendorf tube was vortexed for 5 seconds and the solvent was left to evaporate. Once the sample was dry 100 μL of ethanol was added and the sample briefly vortexed again. At this stage the samples were sealed and placed back into the freezer at -25 °C ready for fluorescence analysis.

The fluorescence analysis was originally planned to be carried out using a fluorescence microplate reader. However an alternative form of analysis had to be devised due to Covid-19 pandemic restrictions. Instead it was decided to adapt a technique used for forensic imaging which utilises fluorescent light sources. A Canon EOS M5 digital camera was used with a Canon EF-M 55-200mm f/4.5-6.3 IS STM lens at 200mm to capture the images. To ensure standardised exposure across all frames, manual mode was used with the following settings: shutter 1/15th second, aperture f/6.3 and ISO 800. The camera, sample and light source were all located in a fixed position, in a completely darkened environment for the duration of the imaging ensuring that the only differences in the image brightness would be due to the varying amounts of fluorescent material within the samples. As each dye has a slightly different excitation wavelength the colour of the light source and the high-pass filter placed in front of the camera varied. For basic yellow a violet light source was used with a pale yellow high-pass filter, and for rhodamine B a blue-green light source was used with an orange high-pass filter. The light sources used for this image analysis were Crime-Lite forensic light sources manufactured by Foster-Freeman (UK). The high-pass filters used in conjunction with these light sources were those designed to accompany each light source. Figure 7.5 shows the imaging setup used for analysis.

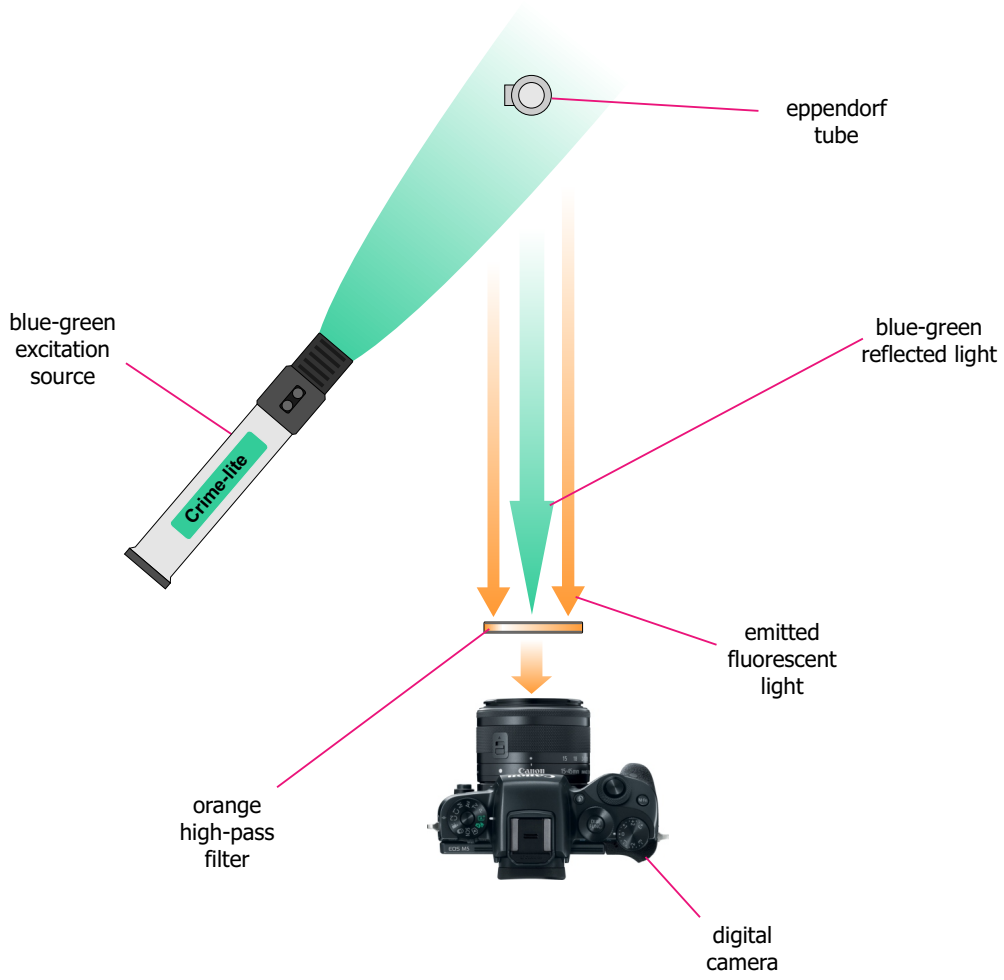


Figure 7.5: Schematic showing the imaging setup used for capturing the fluorescence of each sample. Note that in this schematic there is a gap between the high-pass filter and the camera. This is to show how the high-pass filter works in blocking out all the incident light and allowing only the weak fluorescent light to pass through. In reality this high-pass filter was attached to the camera.

Once all the images had been acquired a series of post-processing steps was carried out to standardise and process each of the images. Firstly each image was cropped to 174 x 174 pixels so that just the centre of the Eppendorf tube was included. Following this, each image was converted to black and white. For both of these processes the photographic software Adobe Lightroom was used (Adobe Inc. US). Finally each image was opened in Adobe Photoshop (Adobe Inc. US) and its brightness histogram displayed. From each histogram the mean brightness level of the image was recorded in a spreadsheet, along with its associated standard deviation. The mean brightness level is a measurement which describes the brightness of an image; a brightness value

of 0 corresponds to true black, whilst 255 is brightest level and therefore represents true white. Therefore the higher the mean brightness level, the brighter the image. The flow chart shown in Figure 7.6 illustrates the steps that were carried out and an example of the post-processing work flow.

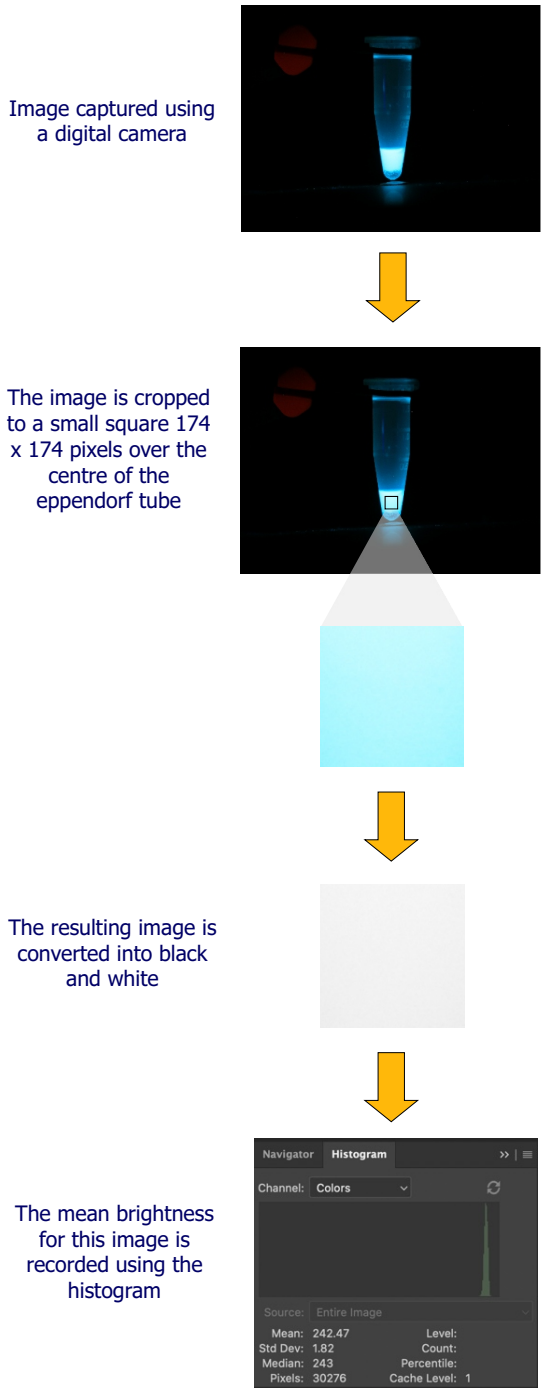


Figure 7.6: Flow chart showing the steps involved in processing each of the sample images. The example shown here is for sample number 18 from the third repeat of the 1 g/L basic yellow dye and *Formica lemani*. As it can be seen, this sample has a mean brightness level of 242.47.

Once the data collection process described above was carried out for each image, a rank

was assigned and recorded in a table with the highest mean brightness assigned a rank of one. In addition to this each sample was ranked according to the amount of isotopic incorporation that was seen based on the hydrocarbon analysis. Hence each sample had two ranks assigned based on how bright its fluorescence was and how much incorporation was observed. These two ranks were analysed firstly by plotting a scatter graph; this is a very simple way to visually show a positive or negative correlation between paired samples. To further analyse any subtle relationships, Spearman's rank-order correlation (r_s) was calculated for each data set, along with an associated probability value. Spearman's rank-order correlation (r_s) is a non-parametric statistical analysis technique used to compare two groups in order to determine if there is correlation between the two. When performing the calculation a value for (r_s) is generated. If perfect negative or positive correlation is observed between the groups, then the value of (r_s) will be -1 or +1, therefore the closer to 0 the less correlation between the two groups. As before, the associated probability value indicates a statistically significant correlation if p is ≤ 0.050. In terms of the value of (r_s), any negative values can be disregarded. This is because positive correlation suggests that as the amount of fluorescence demonstrated increases the amount of incorporation increases too, which is the theory that this experiment is designed to test. Both of these statistical analyses were performed using SPSS (IBM, US).

7.3 Results & Discussion

7.3.1 Basic yellow

The two species which were tested using this fluorescent dye were *Formica lemani* and *Myrmica rubra*. For all data sets a scatter graph will be presented alongside values for (r_s) and p, calculated using SPSS (IBM, US). Please refer to Appendix E for the raw mean percentage abundance data and Appendix F for images of the fluorescence for each sample.

7.3.1.1 *Formica lemani*

In general *Formica lemani* showed far higher levels of fluorescence compared to *Myrmica rubra* for the same dye at the same concentrations. However the relationships between species and between dyes will be further explored within the discussion section of this chapter. Generally for this species there were plenty of samples; this species proving itself to be very tolerable for this kind of experiment.

1 g/L Dye Concentration

The first of the repeats for this concentration demonstrated a fairly narrow range of data points for both mean brightness and mean percentage abundance. The resulting scatter graph shown in Figure 7.7 shows a fairly random distribution of data points indicating that any correlation between the two variables is highly unlikely. This relationship was further tested via Spearman's correlation; ($r_s[30]$ = -0.202, p = 0.283).

The second of the repeats for this dye concentration again featured a large number of samples. Within the fluorescence measurements there was one sample, sample 28, which showed a much lower level of fluorescence compared to the others. The reason for this is not known, however possible reasons will be discussed at the end of the chapter. More interestingly is that this sample also showed the lowest value for mean

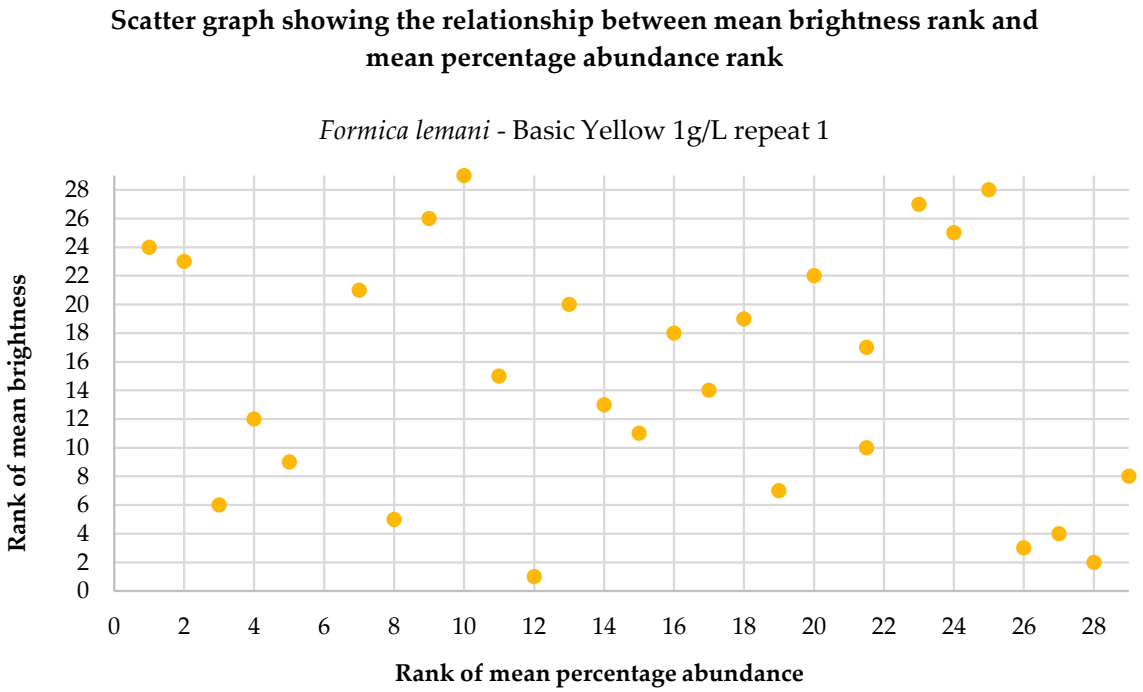


Figure 7.7: Scatter graph showing the correlation between the mean brightness ranks and the mean percentage abundance ranks for the paired samples for *Formica lemani* and basic yellow, repeat 1, 1 g/L.

incorporation, however not to the same extent as that shown for the brightness, see Appendices E.3-E.4 and F.2. The resulting scatter plot of this data does not indicate any correlation despite the observation described above, see Figure 7.8. The lack of correlation between variables was subsequently confirmed via statistical methods; ($r_s[29] = 0.139$, $p = 0.472$).

For the final repeat it was again observed that the sample which exhibited the least amount of fluorescence also showed the least incorporation of the labelled substrate, see Appendices E.5-E.6 and F.3. However despite this relationship there was no correlation shown; ($r_s[29] = 0.107$, $p = 0.581$), see Figure 7.9.

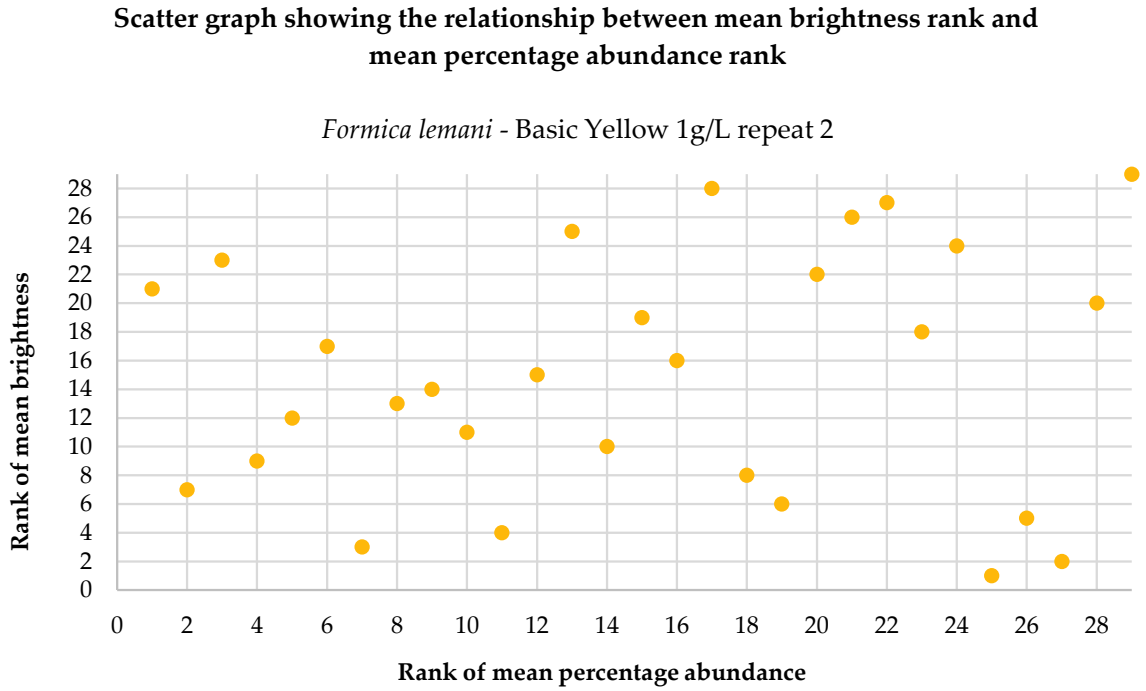


Figure 7.8: Scatter graph showing the correlation between the mean brightness ranks and the mean percentage abundance ranks for the paired samples for *Formica lemani* and basic yellow, repeat 2, 1 g/L.

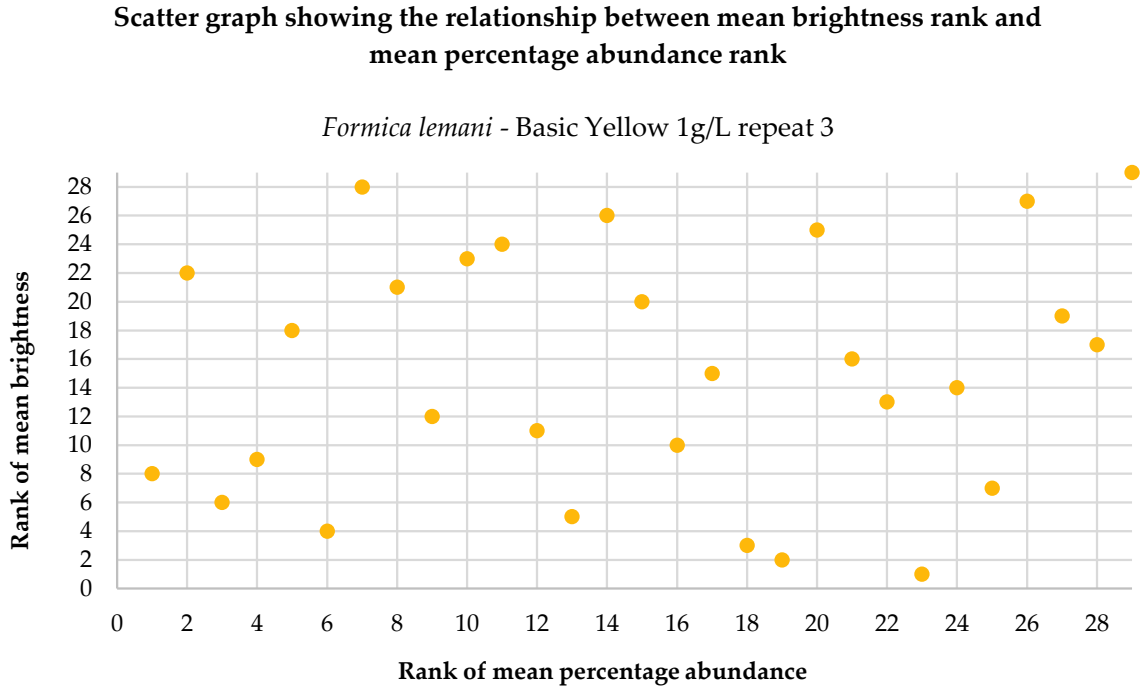


Figure 7.9: Scatter graph showing the correlation between the mean brightness ranks and the mean percentage abundance ranks for the paired samples for *Formica lemani* and basic yellow, repeat 3, 1 g/L.

2 g/L Dye Concentration

Despite the increase in dye concentration there did not appear to be any effect on the mortality of the studied species and the number of individuals per repeat remained high for this dye concentration.

For the first repeat there was quite a lot of variation with regards to the amount of fluorescence shown by the samples, with brightness levels varying from 176.51 to 244.11. However there was far less variation exhibited for the substrate incorporation with a very narrow range of mean values of just 19.71% to 22.97%. This suggests that for this species there was very little incorporation observed with these values just slightly greater than those observed for the dye only control. This is an interesting observation as it suggests that despite the fact that the food was consumed by the ants, as evidenced by the fairly intense fluorescence, there was very little subsequent uptake of the labelled substrate resulting in very little incorporation of labelled substrate into the biosynthesised hydrocarbons. This observation will be further explored within the discussion at the end of this chapter. As therefore expected the scatter graph, Figure 7.10, and the statistical analysis showed no correlation ($r_s[25] = -0.063$, $p = 0.759$).

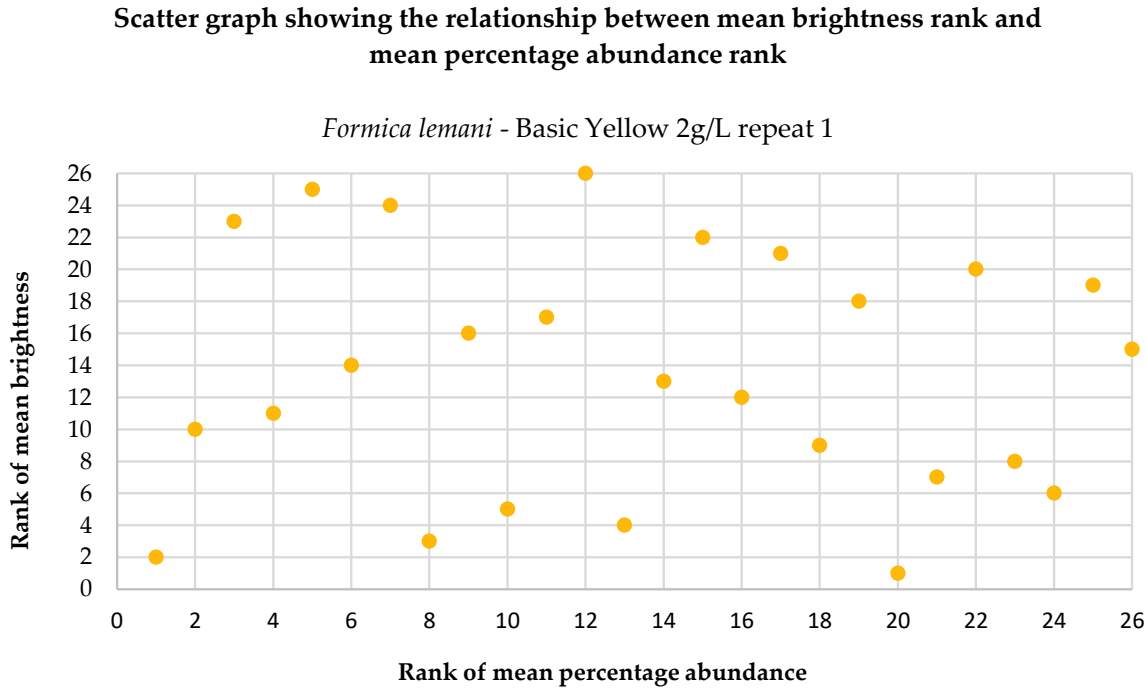


Figure 7.10: Scatter graph showing the correlation between the mean brightness ranks and the mean percentage abundance ranks for the paired samples for *Formica lemani* and basic yellow, repeat 1, 2 g/L.

The second of these repeats followed much the same pattern; the range for both sets of data was much narrower, with the mean brightness levels showing more variation. Again the incorporation values were very similar to what would be expected for control values. Figure 7.11 shows the scatter graph and for this data set $r_s[27] = 0.193$, $p = 0.335$.

For both the mean brightness and the mean percentage abundance the data for the third repeat showed very little variation with no outliers present. As such the scatter graph showed data points with a random distribution and Spearman's rank-order correlation confirmed a lack of any correlation; $r_s[30] = 0.123$, $p = 0.516$.

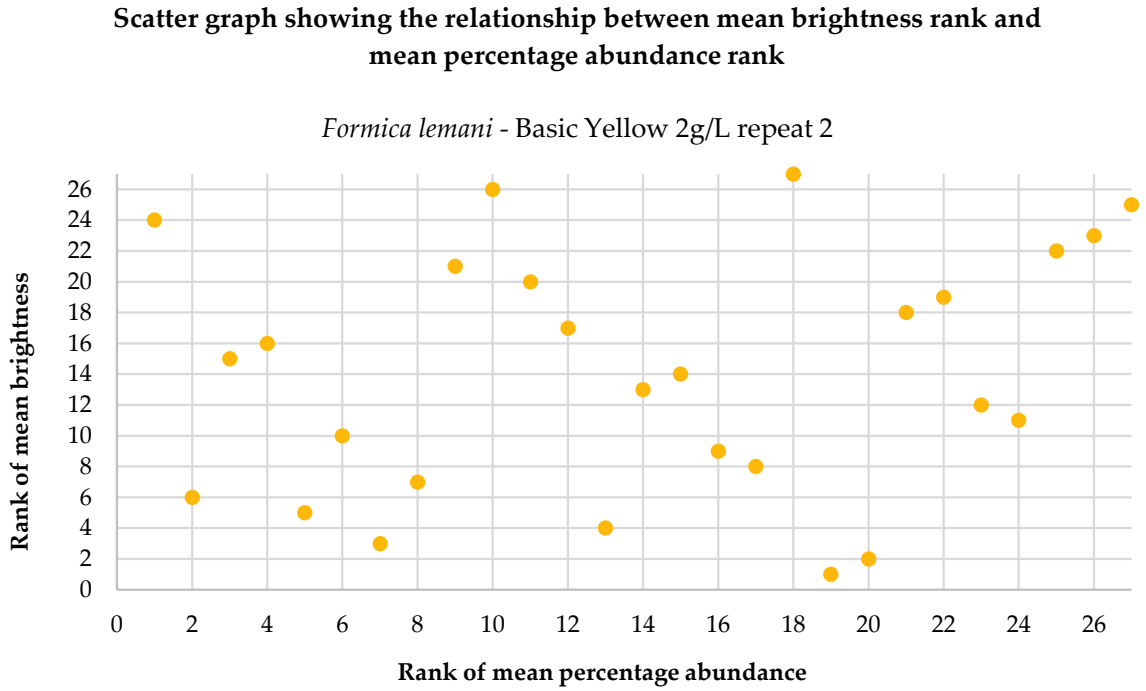


Figure 7.11: Scatter graph showing the correlation between the mean brightness ranks and the mean percentage abundance ranks for the paired samples for *Formica lemani* and basic yellow, repeat 2, 2 g/L.

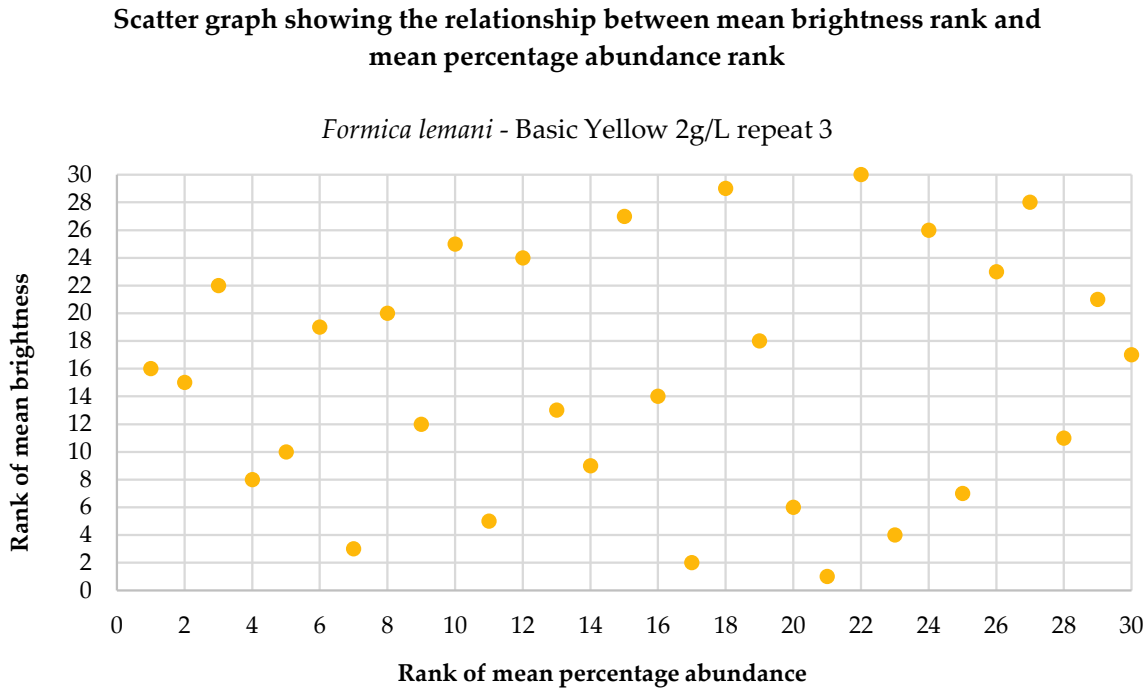


Figure 7.12: Scatter graph showing the correlation between the mean brightness ranks and the mean percentage abundance ranks for the paired samples for *Formica lemani* and basic yellow, repeat 3, 2 g/L.

5 g/L Dye Concentration

The strongest dye concentration to be tested for this species was 5 g/L. For this dye concentration there were only samples remaining at the end of the experiment for repeats one and three. The reasons for this are unknown but will be explored at the end of the chapter when analysing the results further. Despite not having any samples from repeat two, both repeats one and three resulted in a high number of samples suggesting that there was no associated toxicity from the high concentration of this dye.

The first of the three repeats showed a wide variation for the mean brightness levels which can also be seen when looking at Appendix F.7. However as previously observed for this species the levels of incorporation remained low, and for some samples it could not be said with any certainty that there was incorporation of the labelled substrate into the biosynthesised hydrocarbons, see Appendix E.13-E.14. As expected therefore, neither the scatter graph nor the statistical tests revealed any correlation between the amount of food consumed and the amount of labelled substrate incorporated into the cuticular hydrocarbons, ($r_s[26] = -0.310$, $p = 0.123$.)

The third of the repeats for this dye concentration again showed a high number of samples with n=28 for this data set. Within this data set there was one notable observation in that the brightest sample also showed the greatest amount of incorporation, perhaps suggesting a link between the two. However this correlation was not shown by other samples, nor did the visual or statistical representation of the results show any correlation; ($r_s[28] = 0.354$, $p = 0.064$.). See Figure 7.14 and Appendices F.8 and E.15-E.16.

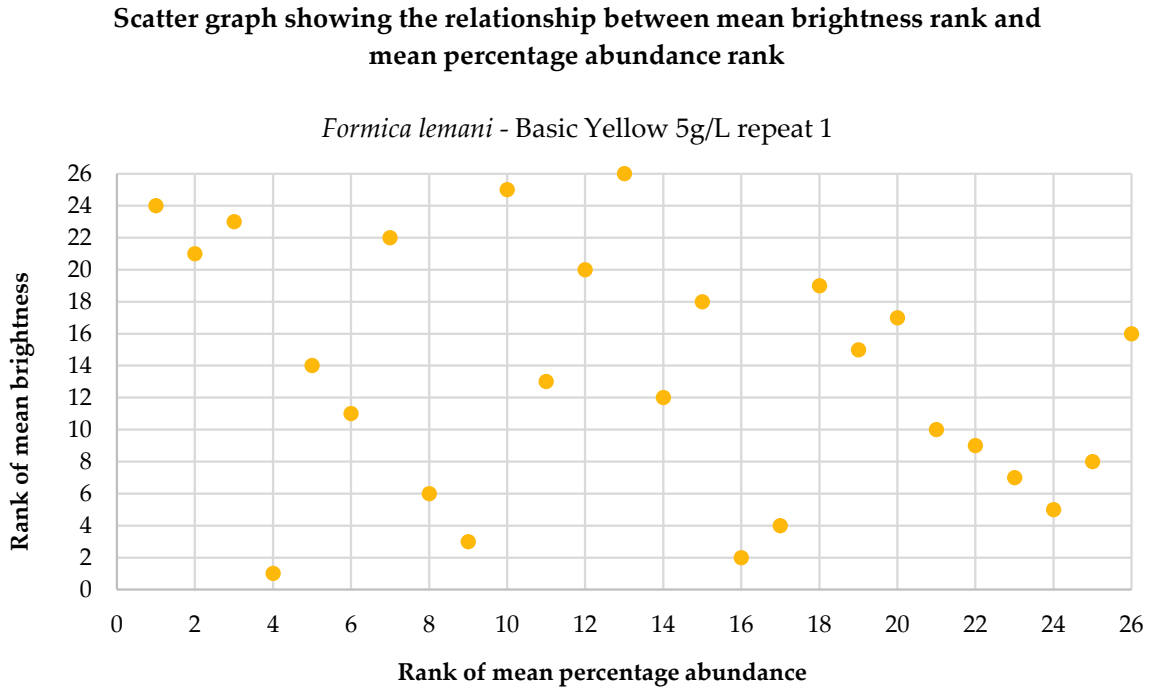


Figure 7.13: Scatter graph showing the correlation between the mean brightness ranks and the mean percentage abundance ranks for the paired samples for *Formica lemani* and basic yellow, repeat 1, 5 g/L.

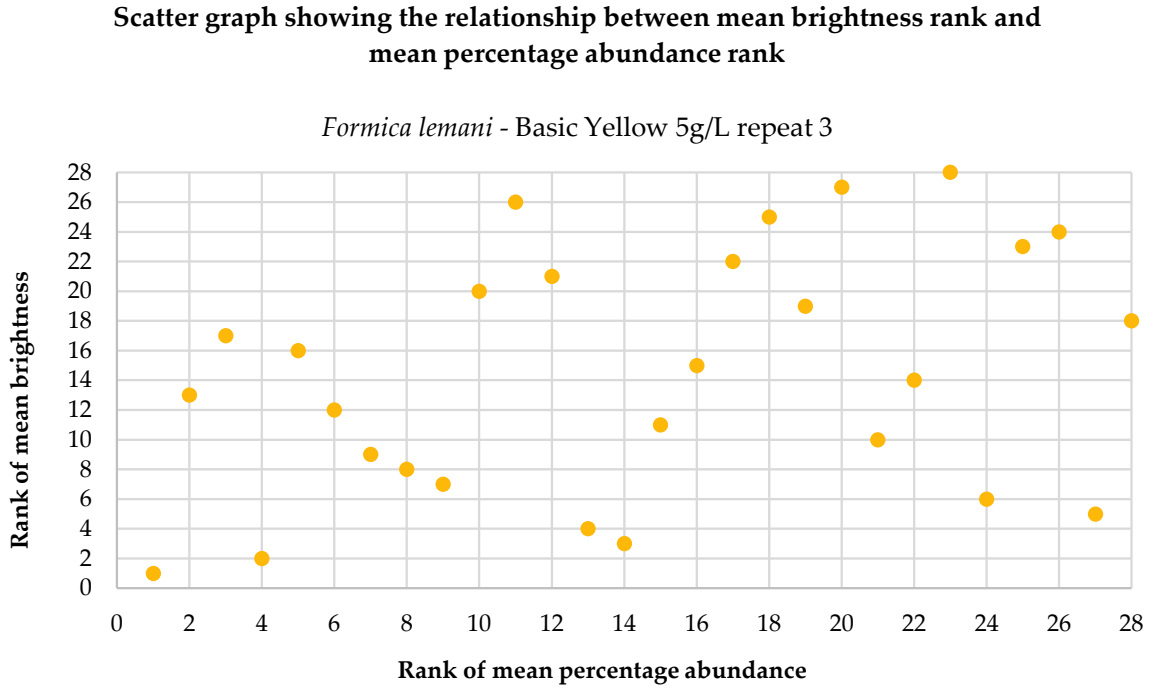


Figure 7.14: Scatter graph showing the correlation between the mean brightness ranks and the mean percentage abundance ranks for the paired samples for *Formica lemani* and basic yellow, repeat 3, 5 g/L.

7.3.1.2 *Myrmica rubra*

1 g/L Dye Concentration

Unfortunately for the first repeat of the 1 g/L dye concentration only four samples were available, therefore this data set was disregarded. For the third repeat there were no available samples at the conclusion of the experiment therefore only the data for the second repeat will be presented. See Figure 7.15 for the scatter graph. As expected, based on the appearance of the scatter graph, the results of Spearman's rank order correlation indicated that there was no association between the brightness of the samples' fluorescence and their corresponding mean percentage abundances, therefore demonstrating that there is no apparent correlation between the amount of food consumed and the resulting isotope incorporation, ($r_s[10] = 0.158$, $p = 0.663$).

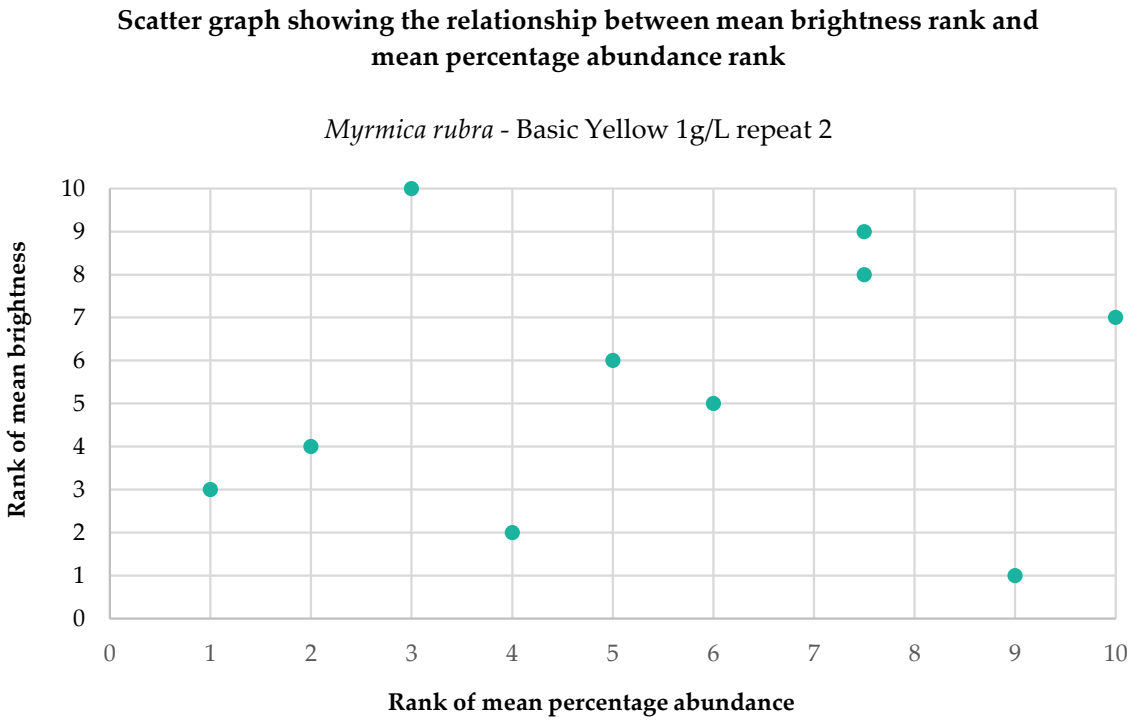


Figure 7.15: Scatter graph showing the correlation between the mean brightness ranks and the mean percentage abundance ranks for the paired samples for *Myrmica rubra* and basic yellow, repeat 2, 1 g/L.

2 g/L Dye Concentration

For the 2 g/L dye concentration of basic yellow there were sufficient samples for all three repeats. The three associated scatter graphs are presented in Figures 7.16, 7.17 and 7.18. For all three sets of paired samples, Spearman's rank order correlation showed that there was no association between the fluorescence of the sample i.e. the amount of food consumed and the resulting isotope incorporation.

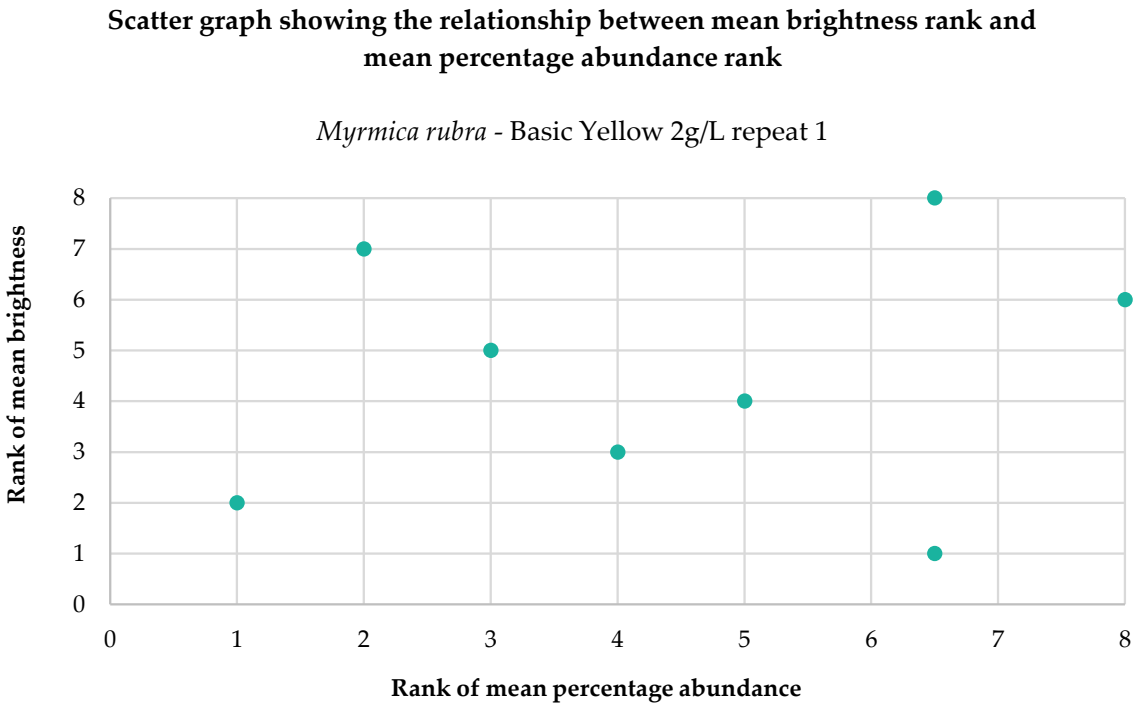


Figure 7.16: Scatter graph showing the correlation between the mean brightness ranks and the mean percentage abundance ranks for the paired samples for *Myrmica rubra* and basic yellow, repeat 1, 2 g/L.

As can be seen from Figure 7.16, the scatter graph showing the data for the first repeat, the points are positioned randomly across the graph, indicating that there is no relationship between the amount of food consumed and the amount of isotopic incorporation. This was further confirmed by Spearman's rank-order correlation; $r_s[8] = 0.180$, and $p = 0.067$.

For the second of the repeats there were slightly more samples (n=17), however Figure

7.17 suggests that there is no apparent correlation between the two variables. As can be seen from Appendix F.14 the resulting images show the brightness of these samples to be fairly consistent, indicating that the amount of dye consumed was fairly similar between individuals. Further statistical analysis showed there to be no correlation between the parameters; $r_s[17] = 0.047$, p =0.859.

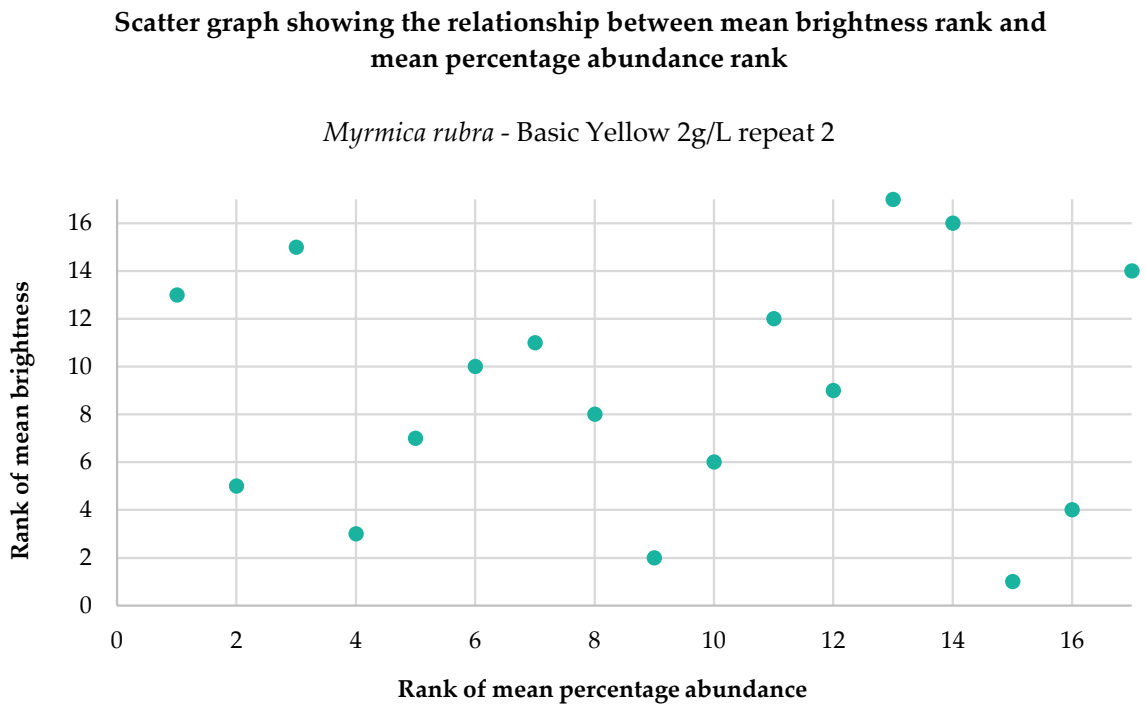


Figure 7.17: Scatter graph showing the correlation between the mean brightness ranks and the mean percentage abundance ranks for the paired samples for *Myrmica rubra* and basic yellow, repeat 2, 2 g/L.

For the third and final repeat at this dye concentration there were many samples for analysis (n=25). Again the brightness of the fluorescence was fairly consistent across the samples, this is in contrast to the isotopic incorporation, see Appendices E.24-E.25 for the raw data sheets, which showed that some samples had larger amounts of incorporation (sample 20 with a mean value of 42.2%) whilst some showed a much smaller amount, (sample 22, just 20.0%). Despite the differences in the amount of incorporation observed there was no correlation between the two parameters, this was confirmed via statistical analysis; $r_s[25] = 0.320$, $p = 0.119$.

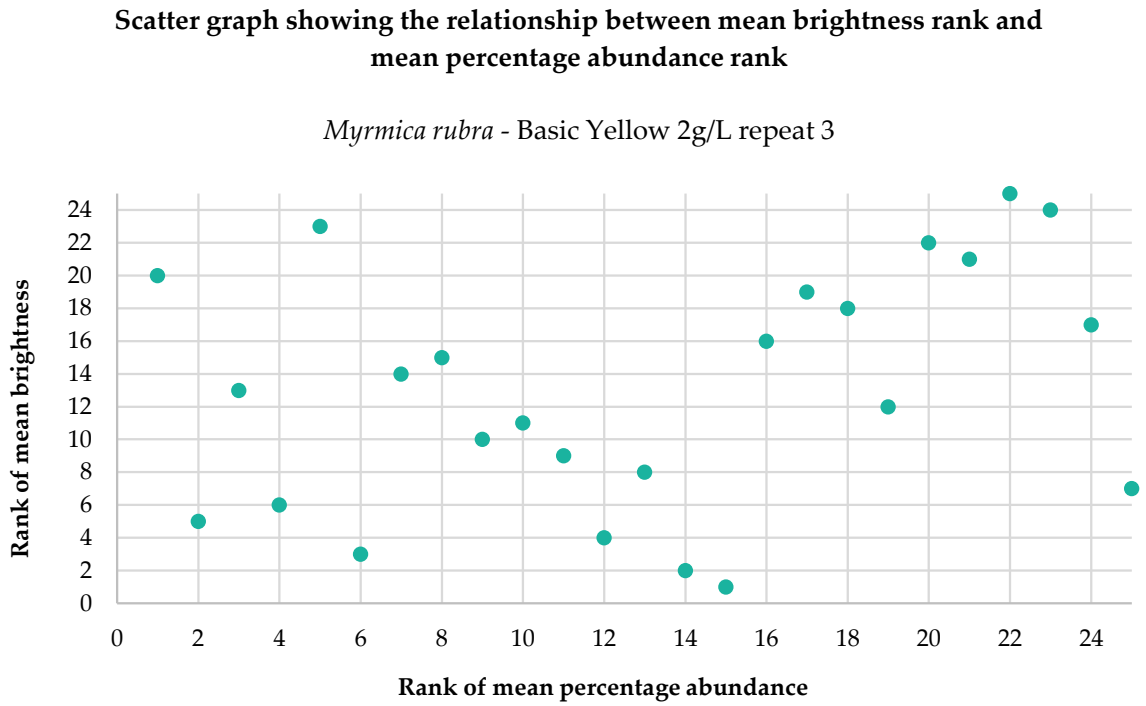


Figure 7.18: Scatter graph showing the correlation between the mean brightness ranks and the mean percentage abundance ranks for the paired samples for *Myrmica rubra* and basic yellow, repeat 3, 2 g/L.

5 g/L Dye Concentration

The final dye concentration to be tested for basic yellow was the strongest; 5 g/L. This high dye concentration was tolerated very well by *Myrmica rubra* with sufficient paired samples for all repeats.

The data for the isotopic incorporation for the first repeat of this sample set shows that the mean incorporation varies from 19.9% to 31.0%, there is therefore some variation demonstrated by individuals in the amount of isotope used in the biosynthesis of the resulting hydrocarbons. In the same way there was considerable variation in the brightness of the fluorescence of each sample, with values ranging from 14.74 to 40.10. However, as Figure 7.19 suggests, there was no discernable correlation between those showing the bright fluorescence and those showing the most isotopic incorporation ($r_s[17] = -0.436$, $p = 0.800$).

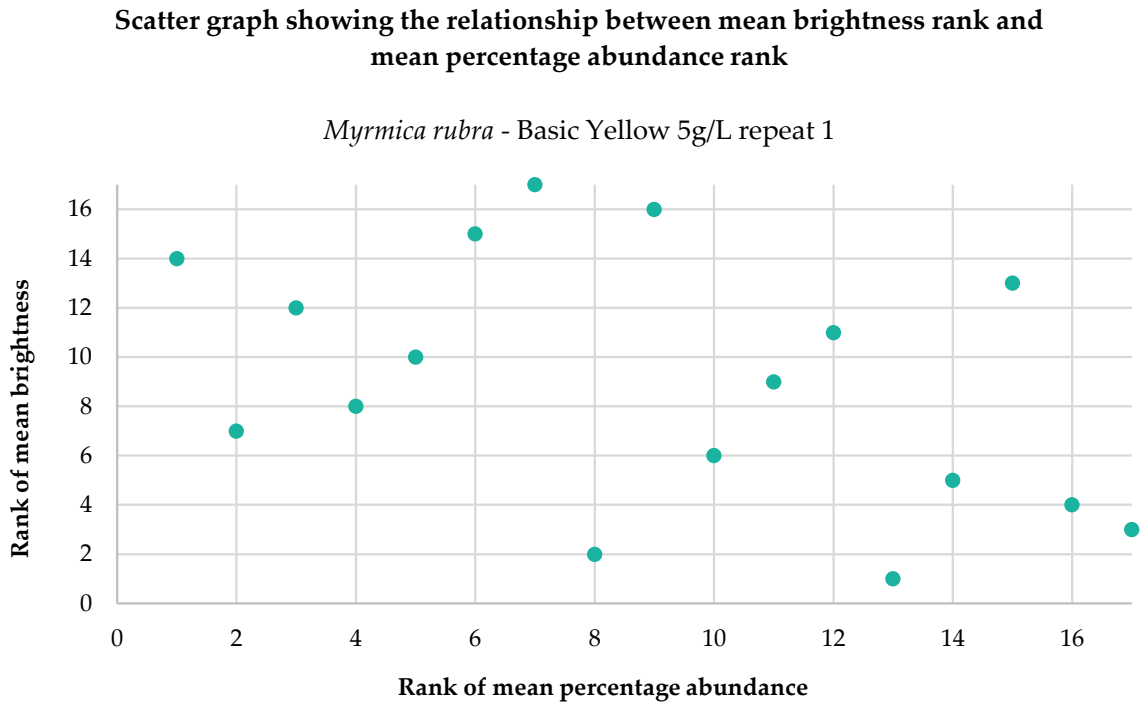


Figure 7.19: Scatter graph showing the correlation between the mean brightness ranks and the mean percentage abundance ranks for the paired samples for *Myrmica rubra* and basic yellow, repeat 1, 5 g/L.

For the second repeat there was a greater number of samples (n=25). In terms of the brightness of the fluorescence there was some variation shown; two samples, numbers 18 and 19, were much brighter than the others (mean brightness values of 87.17 and 79.16 respectively). The brightness of these samples compared to the others within the data set can also be seen by looking at Appendix F.17. However despite the fact that these samples showed far greater levels of fluorescence the amounts of isotopic incorporation were not mirrored against this brightness. Despite this, Figure 7.20, which shows a scatter graph of this data, suggests that there may be some correlation between the two measured variables. However any seemingly positive correlation was not confirmed by further statistical analysis ($r_s[25] = 0.069$, $p = 0.742$).

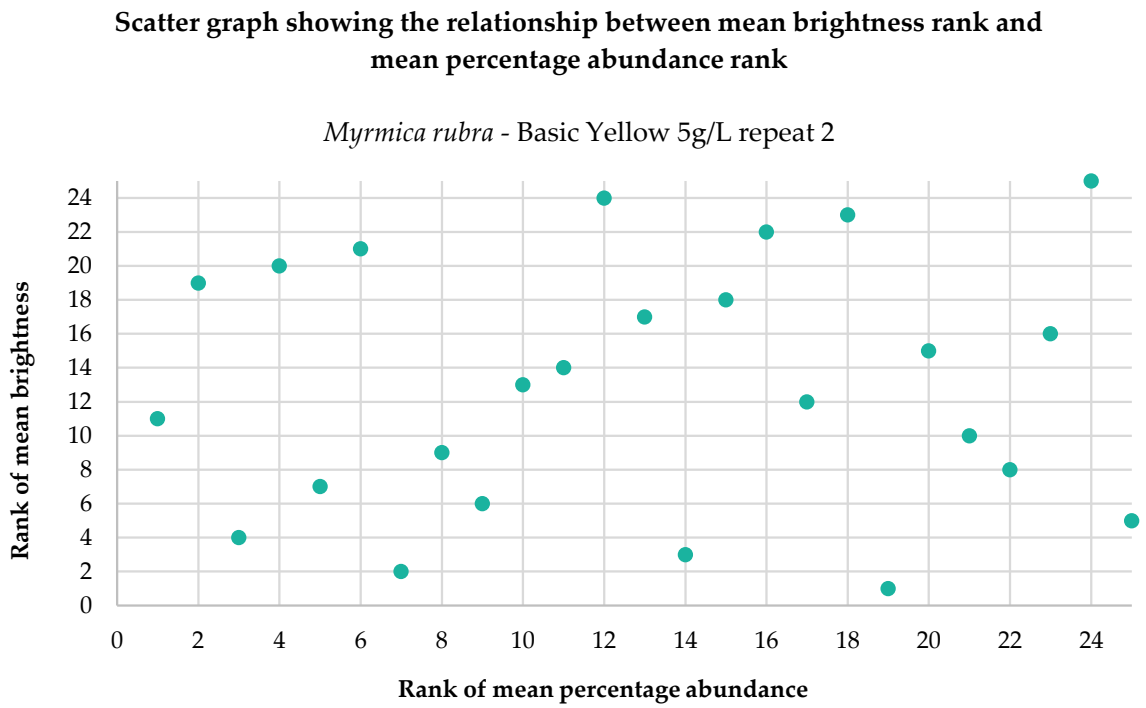


Figure 7.20: Scatter graph showing the correlation between the mean brightness ranks and the mean percentage abundance ranks for the paired samples for *Myrmica rubra* and basic yellow, repeat 2, 5 g/L.

The final repeat for the strongest dye concentration again showed some samples that were much brighter than the others (numbers 13 and 14 within the data set), suggesting that these individuals consumed a greater amount of food than others within the set. However this apparent increase in the amount of food consumed was not reflected in the incorporation for these samples as might have been expected. Figure 7.21 shows the scatter chart for the third repeat, it can be seen that the points appear to be randomly scattered across the graph, an observation confirmed by the results of Spearman's rank-order correlation; ($r_s[24]$ = -0.061, p = 0.778).

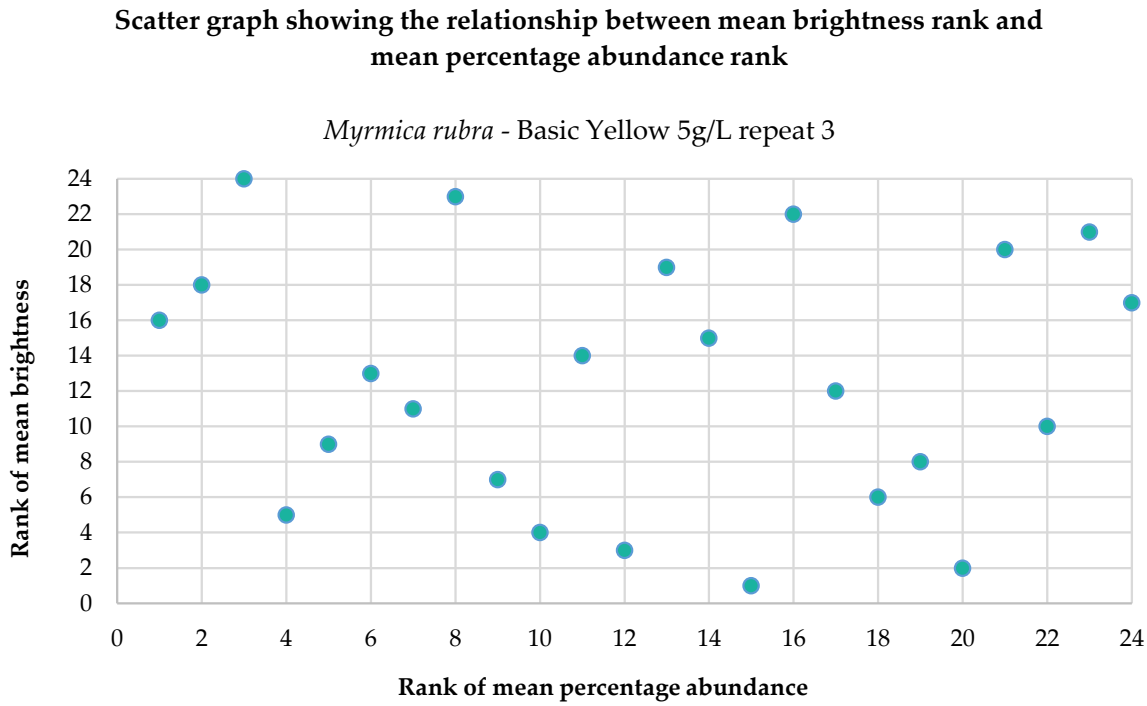


Figure 7.21: Scatter graph showing the correlation between the mean brightness ranks and the mean percentage abundance ranks for the paired samples for *Myrmica rubra* and basic yellow, repeat 3, 5 g/L.

7.3.1.3 Discussion

As this chapter consisted of an experiment that was fairly novel in its design and methods, it is worth at this point considering the variation between the species in relation to the amount of fluorescence and incorporation observed. The aim of this experiment was to determine if there was any correlation between the amount of food consumed and the amount of incorporation of labelled substrate into the biosynthesised hydrocarbons. In previous experiments highly variable amounts of incorporation have been seen within, and between groups, with the amount of physical consumption of the labelled substrate being the most likely reason for this variable incorporation. This experiment was designed to test this theory. Diets containing 1% w/w of the labelled substrate sodium [$^{13}C_2$]acetate were also mixed with varying concentrations of fluorescent dyes. It was theorised that these fluorescent dyes would result in accumulation within the aqueous tissues of the ant and provide a relative measurement of the amount of food consumed. As this experiment was novel in its design it was decided to test two fluo-

rescent dyes, this was in case one of the dyes proved to be toxic. For a similar reason a range of concentrations of dye was tested, it was hoped that if there was toxicity this would only be present at the higher dye concentrations. However the testing of these extra variables allows further analysis of the results, which in turn provides useful insight should this experiment be repeated.

Alongside the three different dye concentrations it was also decided to test a range of controls. These controls consisted of diets containing just the dye at a concentration of 2 g/L and diets containing just 1% w/w of sodium [$^{13}C_2$]acetate. The purpose of the dye only control was to provide control values for the amount of isotopic incorporation. Individuals fed a diet without any ^{13}C labels would provide a comparative baseline for subsequent data analysis, but the presence of the dye would ensure that these controls were fair. In the same fashion the diets containing only 1% w/w of sodium [$^{13}C_2$]acetate and no dye would provide background fluorescence levels, again for comparison purposes.

The chart shown in Figure 7.22 is a comparison of the fluorescence levels of all the tested groups. The values shown for brightness are the average values from within each group, for example the mean brightness for the first repeat for *Formica lemani* at a concentration of 5 g/L was 228.12. This was calculated by taking the mean of the 26 samples that made up this repeat. Note that the controls were only made up of one group; the sodium [$^{13}C_2$]acetate controls are shown in grey and the basic yellow dye only controls are shown in yellow. This graph shows that there are some interesting differences between the various groups.

Firstly it can be seen that the most fluorescence was exhibited by *Formica lemani*, with mean brightness values far higher than those shown by *Myrmica rubra*. There are several possible reasons for this. The first is that *Formica lemani* simply consumed more food, they are the larger of the two species, and therefore it is not unexpected

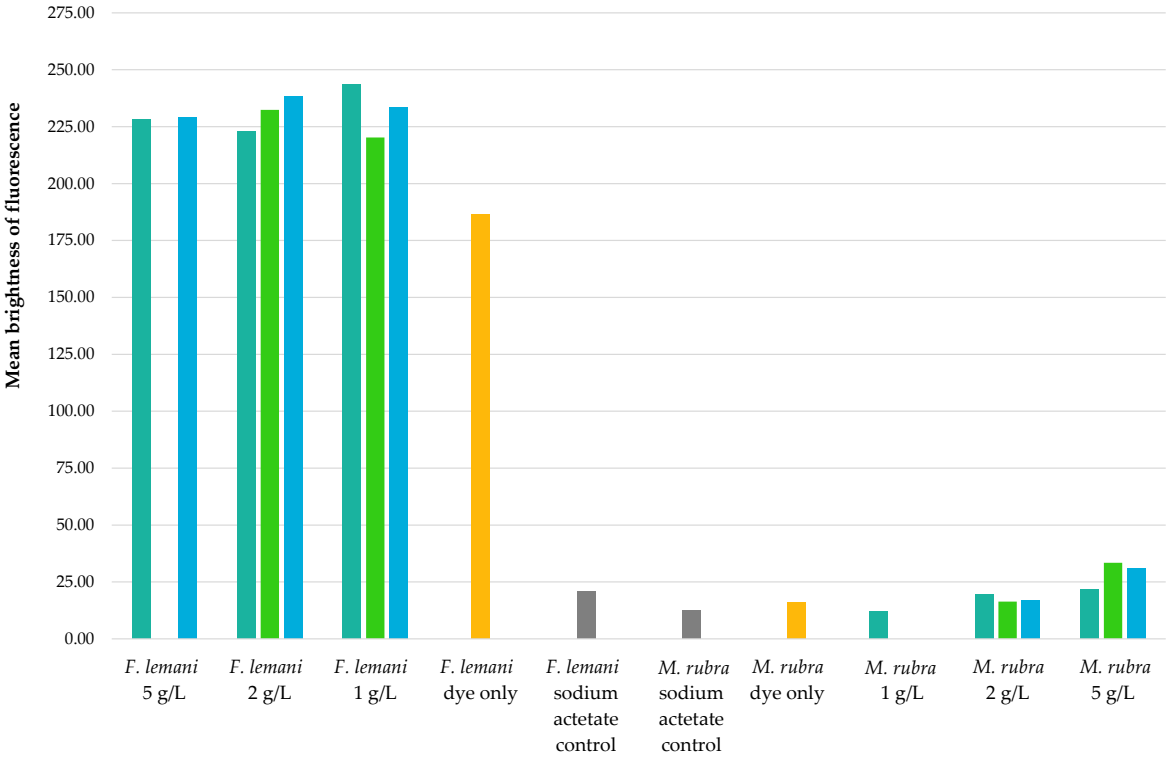


Figure 7.22: Bar chart showing a summary of the mean brightness for each repeat and each of the tested groups for the dye basic yellow.

that they would require more food to sustain their larger physical size. The second reason might be due to the size difference; *Formica lemani* can bio-accumulate a larger amount of dye within its body due to its larger size, compared to the smaller body volume of *Myrmica rubra*. Given the differences in magnitude of the fluorescence shown by the two species the reason for this is more likely to be due to the differing body volumes. *F. lemani* is larger than *M. rubra* and has a much bigger abdomen, hence its body volume is greater, and specifically the volume of haemolymph contained is larger. Haemolymph is the circulatory fluid of the ant and this fluid is likely responsible for accumulating the majority of the dye. Therefore a greater volume of fluid means a greater amount of dye can be accumulated, and therefore a higher dye concentration within each sample results.

Having said this, this may be an overly simplistic view of the relationship. Looking more closely at the graph shows that there is no discernable difference in the mean

fluorescence values for the varying concentrations for *Formica lemani*. This might suggest that there is a maximum bio-accumulation of the dye that is possible. This dye is aqueous in nature and therefore it is highly likely that as well as being accumulated into the tissues it is also able to be excreted from the ant's body. The similar nature of the mean brightness values seen for this species across the varying dye concentrations might represent a steady-state of fluorescence. This might suggest that for this species, lower concentrations of dye would have been more suitable to enable varying levels of dye to have been absorbed, providing a greater variation in the brightness. It is also worth mentioning that the similar brightness levels observed here were not due to the maximum limit of detection being reached by the photographic equipment. In photography maximum brightness, i.e. every pixel being assigned true white, would be indicated by a mean brightness value of 255. So whilst the resulting photographic images were bright, this uniformity is due to consistent levels within the samples, not the technique reaching its maximum detection.

Within the individual *Formica lemani* groups there are some samples which are not as bright than others. These individual samples can be seen in Appendix F which shows the resulting fluorescence images. On occasion the lowest fluorescence values corresponded to the lowest percentage abundance values. However overall correlation between the two variables was not shown across the group by the statistical tests. This suggests that this observation might be due to the individual ant. For example these individual ants could have been in poorer health than the rest of the group, or their health could have deteriorated during the experiment, this could have led to lower metabolism and hence less food consumed and less incorporation shown.

It can be clearly seen that the brightness values for the dye-only control for *F. lemani* is lower than that for the diets containing sodium [$^{13}C_2$]acetate. The dye-only control contained the dye at a concentration of 2 g/L and therefore the brightness levels should be similar to that observed for the 2 g/L substrate diet. However this is not

the case. This may be due to the presence of the sodium acetate; it might be that the salty nature of this compound causes an increased desire to consume the food due to a preferred taste.

When comparing the levels of brightness observed for the sodium [$^{13}C_2$]acetate controls it can be seen that these samples exhibited fluorescence. Each sample imaged consisted of 100 μL of ethanol contained within a plastic Eppendorf tube. During the imaging of these samples it was noted that the high energy violet light source used to promote fluorescence of this dye was causing background fluorescence of the Eppendorf tube. It is this background fluorescence that is responsible for the measureable levels of fluorescence shown by the sodium [$^{13}C_2$]acetate controls. As these controls did not contain any dye the brightness should have been negligible, however this was not observed.

However this only partially explains the differences seen within the data. If the fluorescence levels seen for the sodium [$^{13}C_2$]acetate controls were purely down to background fluorescence then it would be expected that the values for *Formica lemani* and *Myrmica rubra* would be similar. However this is not the case; the values for *Formica lemani* are consistently higher than those for *Myrmica rubra*, in particular there is one sample within the *F. lemani* group that exhibited a mean brightness of 37.6, far higher than expected baseline values of 12-14. The most likely explanation for this is cross contamination between groups during feeding. Although care was taken to thoroughly clean the spatula between groups it is possible that some residual dye remained on the spatula and was transferred into the *F. lemani* sodium [$^{13}C_2$]acetate control group.

In the same way the results observed for *Myrmica rubra* should be scrutinised. For *Formica lemani* it was seen that the bio-accumulation of the fluorescence appeared to reach a saturation point as the mean brightness levels were consistent across all three dye concentrations. However for *M. rubra* a gradual increase of fluorescence was observed as the dye concentrations increased. Although an increase was observed, the

mean brightness levels for this species were far lower than those observed for *Formica lemani*, possible reasons for this have been suggested at the start of this section. The gradual increase between concentrations suggests that the maximum accumulation of dye was not reached for this species suggesting that a higher dye concentration, or longer experiment length could perhaps have been used. It is reassuring to see that there was no real difference between the amount of fluorescence seen for the 2 g/L dye control and the 2 g/L experimental diet which contained the same concentration of dye and sodium [$^{13}C_2$]acetate.

The results of the fluorescence measurements only constitute some of the analysis. In the same way it is worth further analysing the amount of incorporation seen for each species. Figure 7.23 shows a chart comparing the mean percentage abundances for each repeat. These values were calculated in the same way as those for the mean brightness.

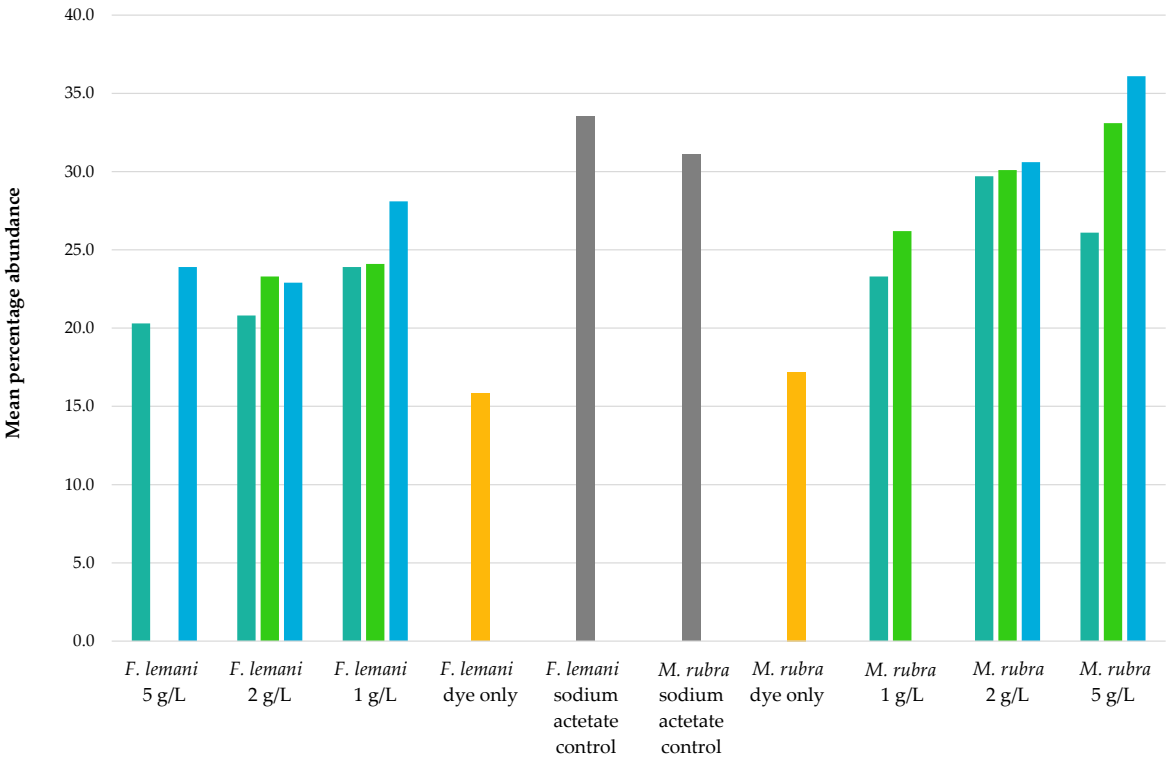


Figure 7.23: Bar chart showing a summary of the mean percentage abundances for each repeat and each of the tested groups for the dye basic yellow.

Again the graph reveals some differences between species. It would be reasonably expected that the abundances for all diets containing sodium [$^{13}C_2$]acetate should be very similar. However it can be seen that this is not the case. The two yellow bars represent the diets which did not contain any isotopic labels and therefore these represent natural, or baseline levels, and are similar between species. Both species show that when consuming a diet labelled with sodium [$^{13}C_2$]acetate there was an increase in the amount of ^{13}C present in the biosynthesised hydrocarbons. This is an entirely expected result. However there is a difference between the amounts of incorporation shown between species, with *M. rubra* demonstrating greater amounts of isotopic incorporation than *F. lemani*. This is similar to trends observed previously which suggest that different species incorporate different levels of labelled substrates, see Chapter 3 for a more detailed discussion of this concept.

It is worth mentioning that whilst *M. rubra* show the higher levels of incorporation, they show lower levels of fluorescence. A previously suggested reason for the difference in brightness seen was theorised at possibly being due to the body volumes of the two ants; *F. lemani* capable of holding a far greater amount of dye compared to *M. rubra*. But it also may suggest that the small body volume of this ant means that this species is not suited to analysis of this type; the mean brightness levels for all three dye concentrations were not much larger than for the sodium [$^{13}C_2$]acetate control. This may suggest that this species more readily excretes the dye from its body, or that it simply does not have the physical volume for bio-accumulation of the dye. Despite either of these being the case, the labelled substrate was more readily incorporated for *M. rubra* compared to *F. lemani*. Again the reasons for differing amounts of incorporation between different species have been previously discussed.

7.3.2 Rhodamine B

The two species which were tested using this fluorescent dye were *Formica fusca* and *Myrmica rubra*. For all data sets a scatter graph will be presented alongside values for (r_s) and p, calculated using SPSS (IBM, US).

7.3.2.1 *Formica fusca*

The dye rhodamine B was selected because it has been widely used as a fluorescent tracer dye in biological organisms [20–22]. In the study by Greenwald it was found that rhodamine B was non-lethal in the studied ant species, even at concentrations up to 10 g/L [20]. Therefore it was unexpected to see that compared to basic yellow this dye seemed to have a more adverse effect on the health of the ant experimental groups. For analysis of the results a minimum number of 6 samples was required, this was a lower than ideal limit but driven by necessity. Despite this lower limit of samples for *Formica fusca*, out of a possible nine experimental repeats (three repeats per concentration), there were only three groups that remained at the end of the experiment. The high mortality observed will be discussed further at the end of this chapter. All three of the groups that were available for analysis resulted from a dye concentration of 2 g/L, and as such only the results for these experimental groups will be shown for this species.

2 g/L Dye Concentration

The first of these repeats for this species contains only seven individuals. A scatter graph was plotted showing the ranks for both mean brightness and mean percentage abundance for each of the seven paired samples, Figure 7.24 shows this graph.

All seven values for mean brightness fall within a fairly narrow range, whilst the range for the mean percentage abundance varies far more. As the scatter graph suggests there is no correlation, this is confirmed by the statistical analysis; $r_s[7] = 0.393$, $p = 0.383$.

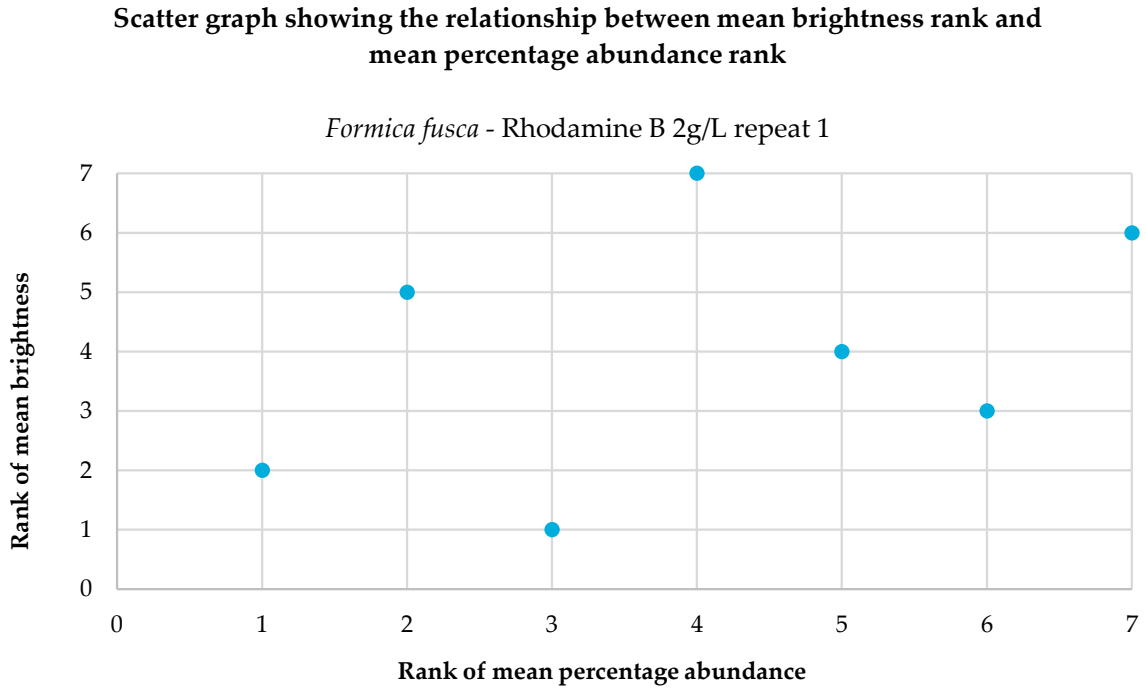


Figure 7.24: Scatter graph showing the correlation between the mean brightness ranks and the mean percentage abundance ranks for the paired samples for *Formica fusca* and rhodamine B, repeat 1, 2 g/L.

For the second of the repeats the data is very similar; there is a narrow range of brightness values and a much wider range of incorporation. Again no real correlation between the six paired samples is shown, Figure 7.25 shows a fairly random distribution of points across the chart and for this data set $r_s[6] = -0.314$, $p = 0.544$.

For the final of the three repeats the number of samples was once again very small (n=6). Again the brightness of the samples was very similar whilst the range of values shown for incorporation was much wider, see Appendices E.34 and F.22 and Figure 7.25. The sample with the highest incorporation of ^{13}C into its biosynthesised hydrocarbons showed one of the lowest brightness values indicating that there was no correlation. This was confirmed via calculations; $r_s[6] = 0.116$, $p = 0.827$.

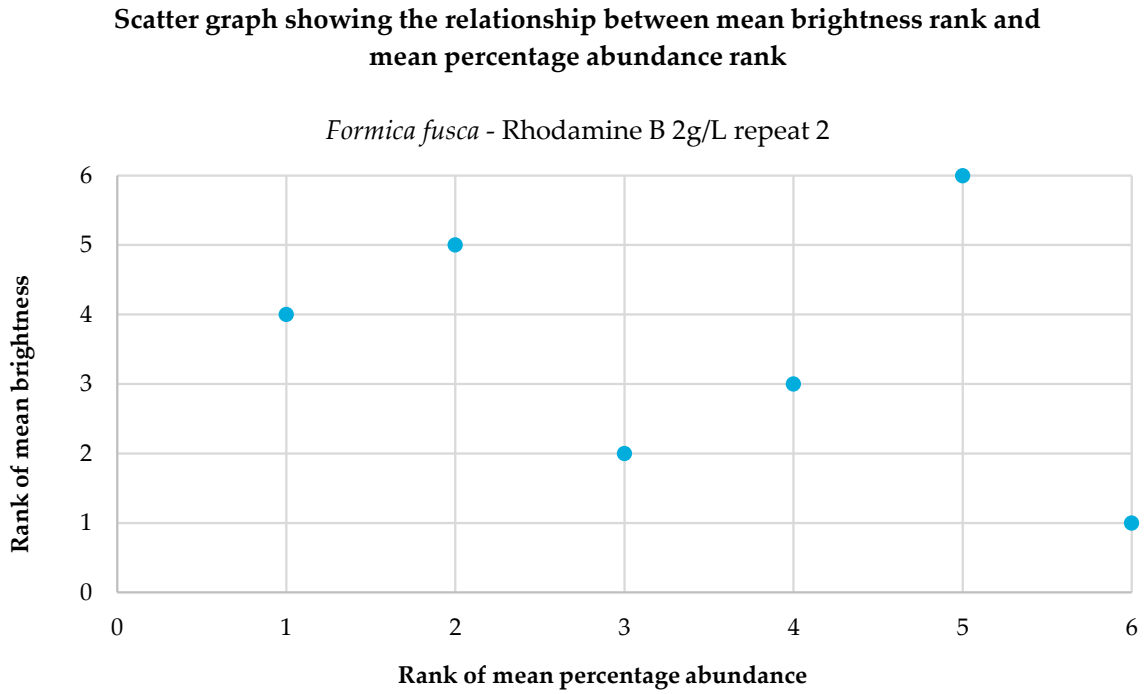


Figure 7.25: Scatter graph showing the correlation between the mean brightness ranks and the mean percentage abundance ranks for the paired samples for *Formica fusca* and rhodamine B, repeat 2, 2 g/L.

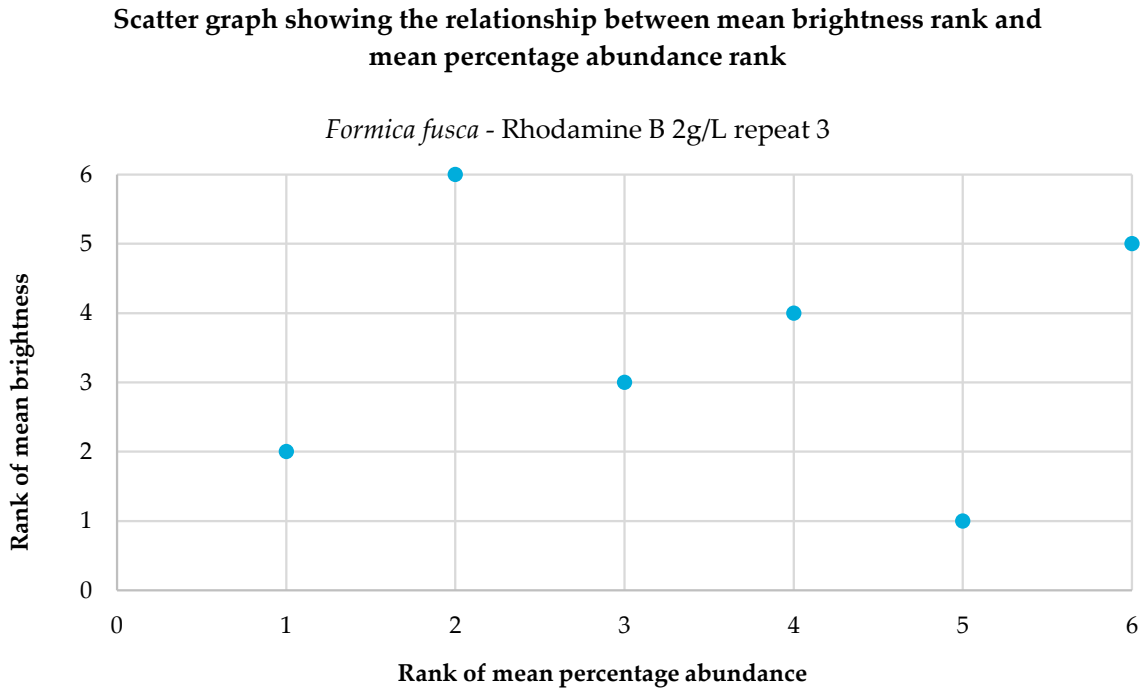


Figure 7.26: Scatter graph showing the correlation between the mean brightness ranks and the mean percentage abundance ranks for the paired samples for *Formica fusca* and rhodamine B, repeat 3, 2 g/L.

7.3.2.2 *Myrmica rubra*

Again for this species the number of useable samples at the end of the experiment was low. Out of a possible nine groups only five groups contained enough samples to be analysed (n $\geq$6). Possible reasons for this will be discussed at the end of this section.

1 g/L Dye Concentration

The number of repeats available for this dye concentration was three. For these three repeats the number of samples in each were 6, 7 and 15, for repeats 1-3 respectively. Each of these three repeats will be analysed via a scatter graph which provides a visual representation of the correlation between paired samples and subsequent statistical calculations.

For the first of the repeats please see Figure 7.27. With just 6 samples within this data set it is extremely difficult to judge any correlation between the paired samples. There is a wide range within the brightness values, from 16.26 to 70.16, however this is not reflected in the abundances of isotope incorporation, and values here range from just 23.05% to 31.77%. If strong correlation was observed between paired samples it would be expected that the brightest sample, number 13, would show a large incorporation, however this is not the case. The results of the statistical calculation confirm this with $r_s[6] = 0.429$, $p = 0.397$.

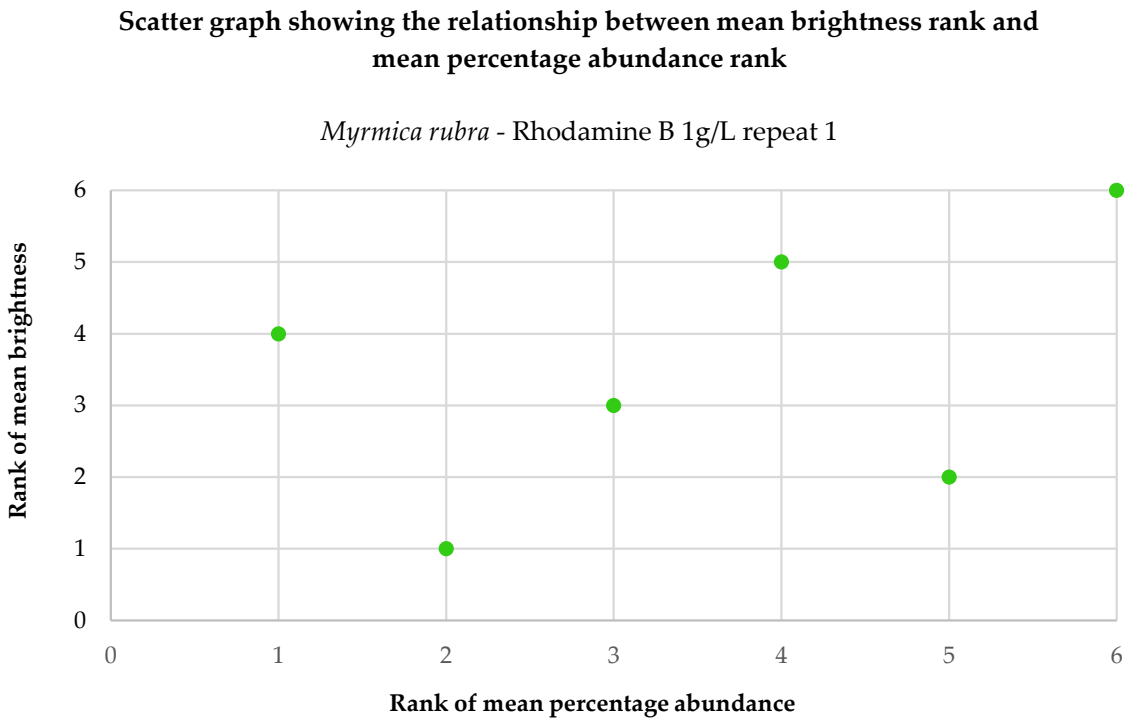


Figure 7.27: Scatter graph showing the correlation between the mean brightness ranks and the mean percentage abundance ranks for the paired samples for *Myrmica rubra* and rhodamine B, repeat 1, 1 g/L.

Again for the second of the repeats the amount of fluorescence demonstrated by the samples within this group varies fairly widely from 28.72 to 56.93, but again all the mean percentage abundance values are very similar with a range covering just 8% difference. With this being a small group of samples it would be easy to judge any correlation visually, however as Figure 7.28 shows there is no observable correlation between samples; $r_s[7] = -0.286$, $p = 0.535$.

The final of the three repeats contained the largest number of samples with n=15. With this data set there was the largest observed variation in the mean values observed so far, with the lowest measured fluorescence for one sample at just 1.58, whilst the highest was 100.86. The difference between brightnesses for these samples can be seen by looking at Appendix F.28, which shows the fluorescence images. The difference between brightness can be clearly seen. With such vastly different brightnesses it might

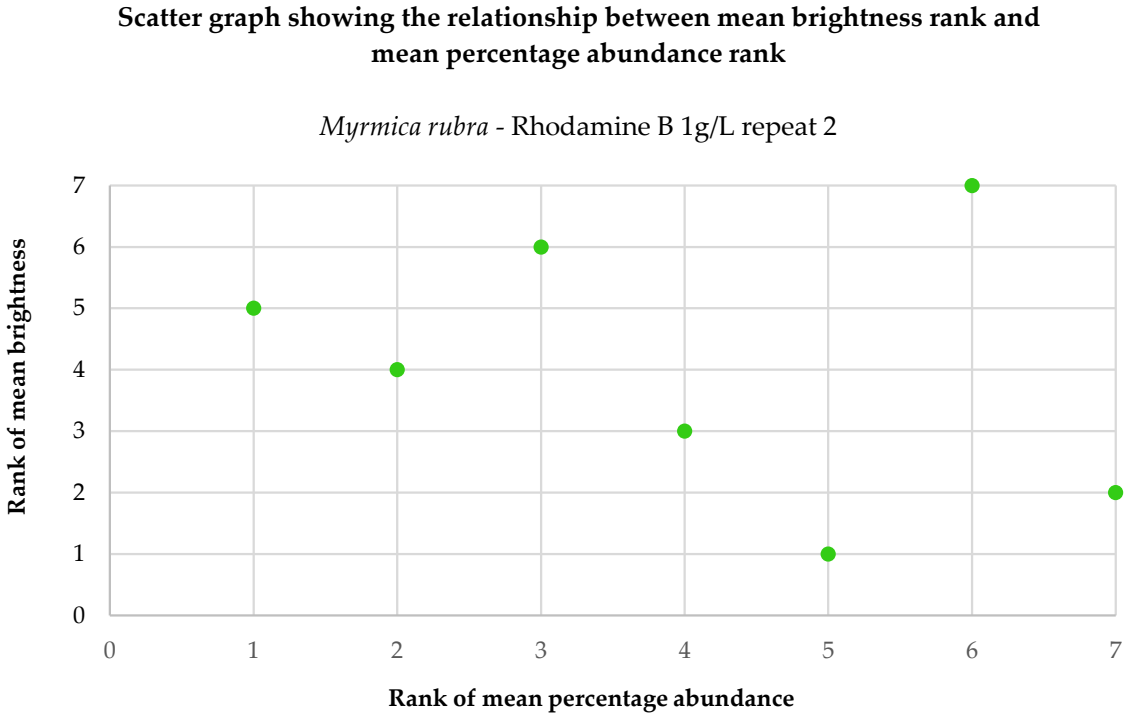


Figure 7.28: Scatter graph showing the correlation between the mean brightness ranks and the mean percentage abundance ranks for the paired samples for *Myrmica rubra* and rhodamine B, repeat 2, 1 g/L.

be expected that the least and greatest amounts of incorporation would correspond to the fluorescence values however this again not the case with no correlation observed, ($r_s[15] = 0.029$, $p = 0.919$.) In fact this value represents the lowest correlation value seen so far.

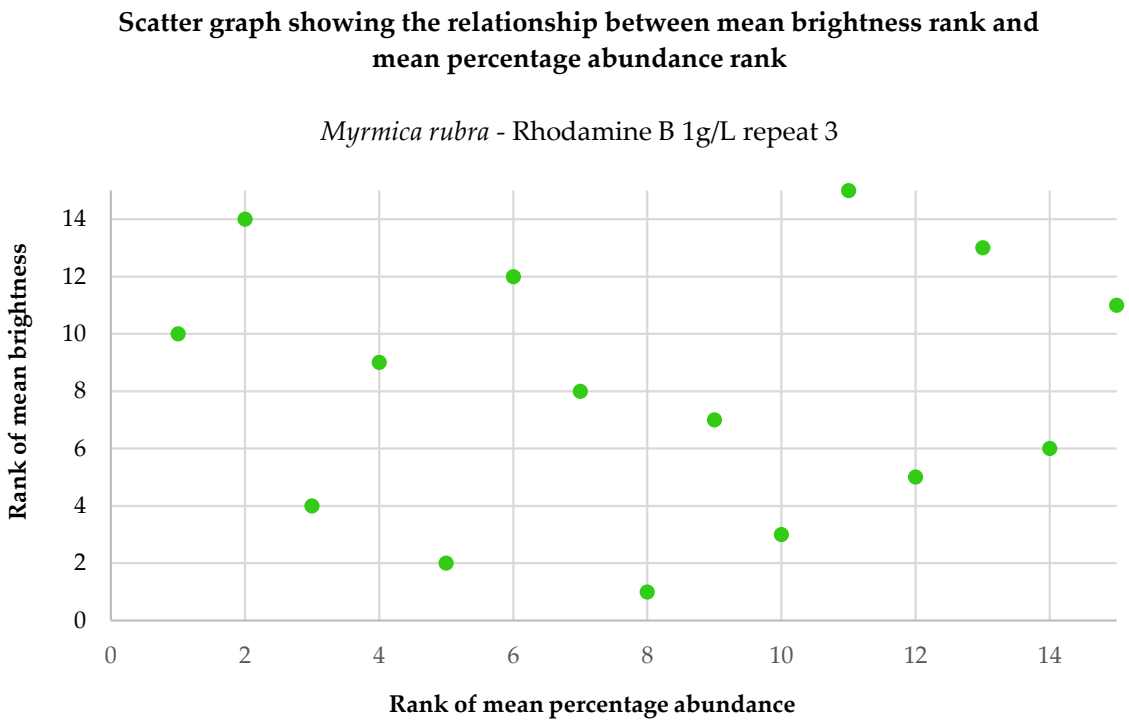


Figure 7.29: Scatter graph showing the correlation between the mean brightness ranks and the mean percentage abundance ranks for the paired samples for *Myrmica rubra* and rhodamine B, repeat 3, 1 g/L.

2 g/L Dye Concentration

At the end of the experiment a total of just six samples remained from the second of the repeats. There was a fairly wide variation seen for the brightness of these samples with values from 9.34 to 44.68 observed. The variation for the percentage abundance values was much smaller, with values from 24.91% to 30.46% observed. Looking at the two sets of ranks for this data set it is possible that there is some correlation between the groups, the highest and the lowest values for each variable are for the same sample. This can be seen by looking at Figure 7.30 and in the higher value for Spearman's Rho ($r_s[6] = 0.600$, $p = 0.208$.) However with just six samples within this dataset this is hardly a reliable assumption.

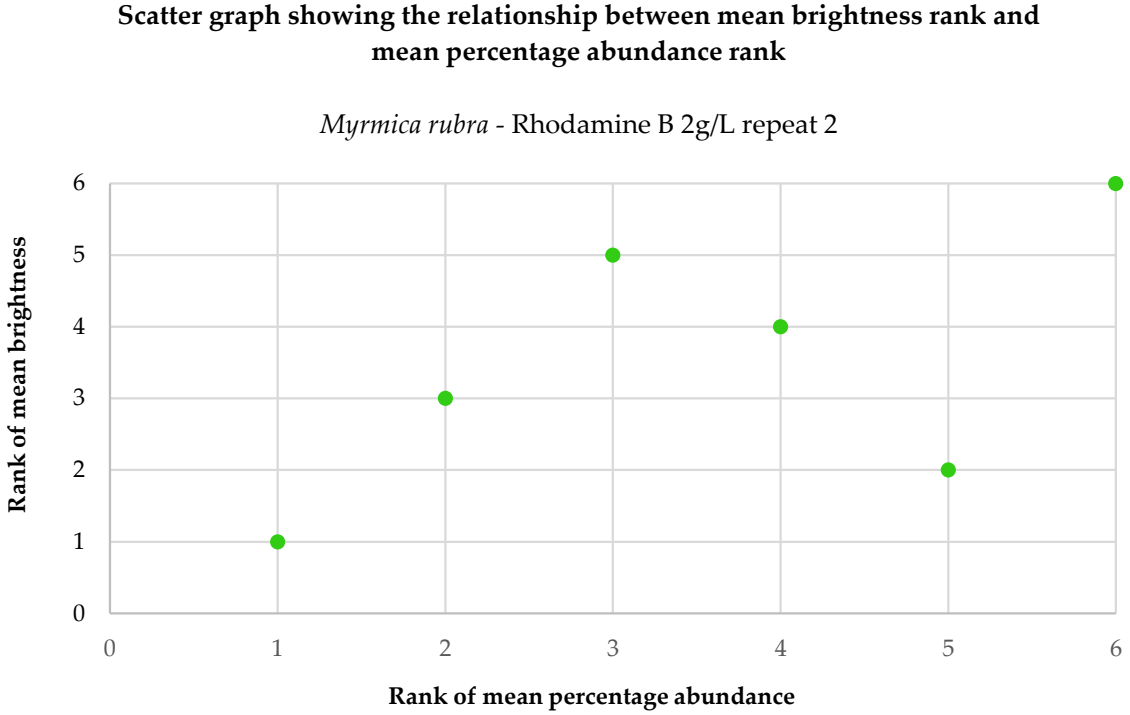


Figure 7.30: Scatter graph showing the correlation between the mean brightness ranks and the mean percentage abundance ranks for the paired samples for *Myrmica rubra* and rhodamine B, repeat 1, 5 g/L.

5 g/L Dye Concentration

The final data set for analysis in this limited dye group is the first repeat for the 5 g/L dye concentration. This group contained nine samples, and whilst there were individuals analysed in the other repeats, the number of samples was considered too small for further consideration. As such only the data for the first repeat will be presented here. Again the situation is similar to that observed for the other groups; there is a wider range of mean brightness values than there are percentage abundance values. Further statistical analysis suggests that there may be some weak negative correlation, however as previously mentioned negative correlation is meaningless for analysis of these results, ($r_s[9] = -0.633$, $p = 0.067$.)

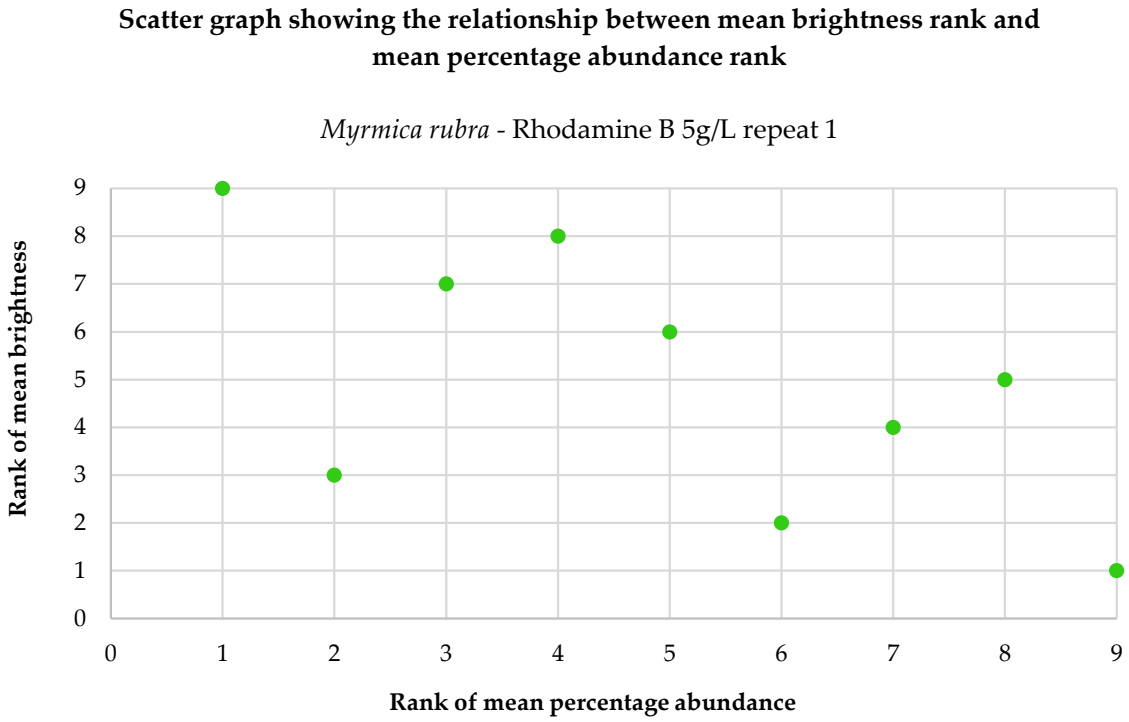


Figure 7.31: Scatter graph showing the correlation between the mean brightness ranks and the mean percentage abundance ranks for the paired samples for *Myrmica rubra* and rhodamine B, repeat 1, 5 g/L.

7.3.2.3 Discussion

When this study was being planned it was decided to test two dyes in case there was a difference in the observed mortality and hence an indication of toxicity. The first of the fluorescent dyes, basic yellow 40 is most commonly used in forensic science. As such its toxicity within ants had not been studied. Conversely the second of the studied dyes, rhodamine B, has been widely used within biological organisms and has been reported as non-lethal to ants even up to concentrations of 10 g/L [20]. Despite the assumption that this dye would be safe for use within our tested concentration range there were noticeably fewer samples for analysis at the end of the experiment for rhodamine B. In total approximately 600 ants were tested with this dye, 300 of each species. At the end of the experiment for *Myrmica rubra* just 69, or 23% of ants remained and for *Formica fusca* there were even fewer at 42, or 14%. This is compared to the numbers

for basic yellow; for *Formica lemani* 261 ants, or 87% remained and for the directly comparable species *Myrmica rubra* 142, or 47% remained. It is not possible to compare differing species, although it does appear based on these numbers that *Formica lemani* is the most robust of the studied species. Based on the directly comparable numbers for *Myrmica rubra* it seems that rhodamine B may have had a negative effect on the health of the individuals.

However if rhodamine B was adversely affecting the health of our studied samples it would be expected that the higher concentrations would have had fewer remaining samples at the end of the experiment and this was not the case. There was no discernable link between the dye concentrations and the numbers of samples. Instead the high levels of mortality may have been due to the extreme environmental conditions that the studied groups were subjected to. This experiment, which ran for approximately 28 days, featured periods of very high laboratory temperatures ($\sim$ 30 °C), and rapid mortality was observed during this period for *Myrmica rubra*. This is likely due to the effects of the high temperature and a lack of water within the experimental containers. Although efforts were made to ensure that the soil substrate remained moist, this had to be balanced against over-hydration and the formation of mould, promoted by the presence of the sugar rich diets.

Despite a lack of correlation being observed for any of the studied groups, analysing the mean brightness results for both species reveals some interesting features, see Figure 7.32.

As this graph shows there are far fewer data sets for comparison. For *Formica fusca* the only data that was possible for analysis was that of the 2 g/L dye concentration. For this data set the three repeats showed remarkably similar values for brightness indicating extremely consistent levels of fluorescence across the three groups. Due to the lack of data for other concentrations it is impossible to know whether this represents the

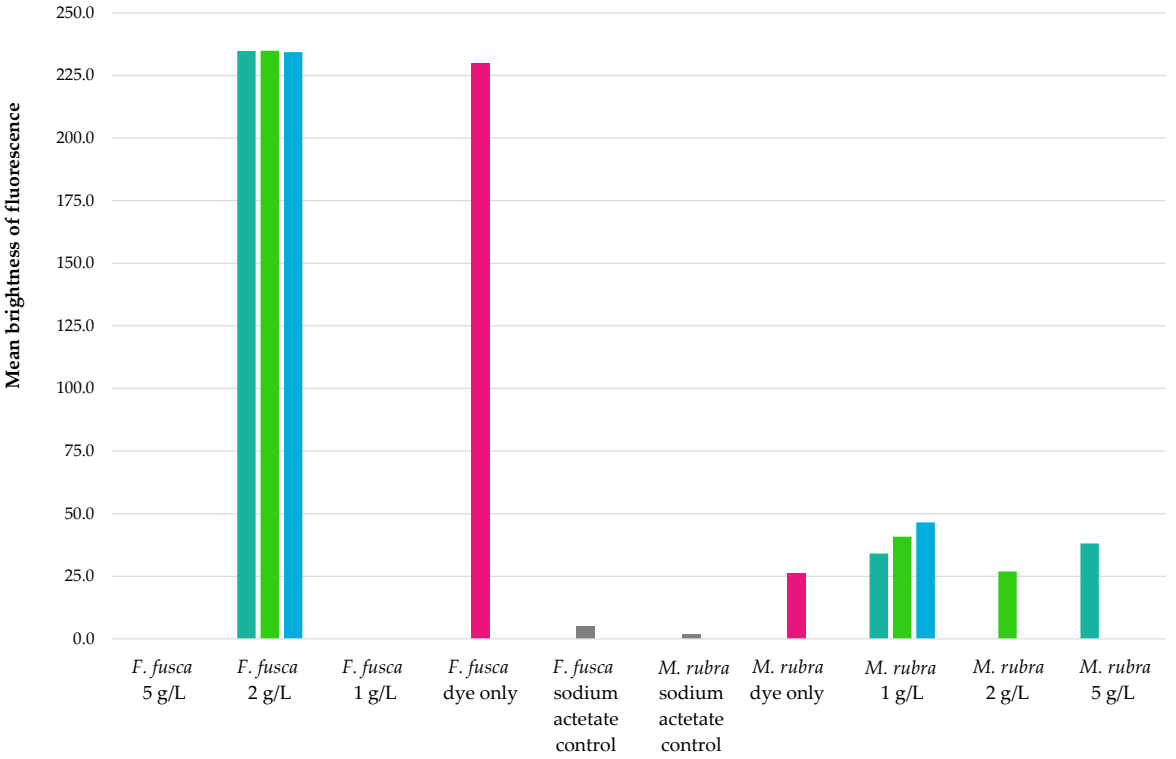


Figure 7.32: Bar chart showing a summary of the mean brightness for each repeat and each of the tested groups for the dye rhodamine B.

maximum fluorescence for this species or whether the higher and lower concentrations would have demonstrated greater and lesser amounts of fluoresence. The brightness of the fluorescence seen for the 2 g/L substrate diet was also very similar to that seen for the dye only control. This is to be expected as the concentration of the dye only control was also 2 g/L.

For basic yellow the violet excitation source used to promote fluoresence caused some background fluorescence of the Eppendorf tubes and hence reasonable fluorescence was seen for the sodium [$^{13}C_2$]acetate controls. These controls did not contain any fluorescent dye however for basic yellow mean brightness levels of 21.0 and 12.7 were observed for *Formica lemani* and *Myrmica rubra* respectively. When the lower energy excitation source was used with rhodamine B there was no background fluorescence observed and hence the recorded brightness levels were negligible; 5.2 and 1.9 for *Formica fusca* and *Myrmica rubra* respectively.

Looking at the mean brightness levels for *Myrmica rubra* it can be seen that there is no real difference between the brightness levels for the different concentrations. In addition the mean brightness levels are far lower than those for *Formica fusca*. Despite the slightly different species the results are very similar to those seen for *M. rubra* and *F. lemani* and basic yellow. This may be for the same reasons as those previously described. *Formica fusca* is a very similar size to *F. lemani* and consequently like *Formica lemani* this species may have a greater capacity to bio-accumulate the fluorescent dye compared to the smaller *M. rubra*.

Finally it is worth mentioning that it is impossible to compare the mean brightnesses between dyes. This is because each dye was analysed using a different combination of excitation source and high-pass filter. Whilst steps were taken to ensure that each combination selected provided the best possible results for each dye, there will have been differences between them. Due to this lack of uniformity between dye imaging conditions, it is impossible to make comparisons between the dyes.

As well studying the data for the mean brightness it is also possible to further study the mean percentage abundance values which represent the amount of incorporation seen for each species. See Figure 7.33 for a visual comparison of the percentage abundance data for the various measured groups.

Again the data for this dye is fairly limited, however it can be seen that the values for percentage abundance are fairly similar across all the repeats for *Formica fusca*. These values are also far higher than the dye only control values which represent a baseline level for comparison. This shows that the labels present within the substrate were incorporated into the resulting biosynthesised hydrocarbons, this is not unexepected, this substrate was chosen as it was known to result in labelled cuticular hydrocarbons. However again there is a fairly large difference in the amount of incorporation between the two studied species. For these species and this dye the largest amount of incorpo-

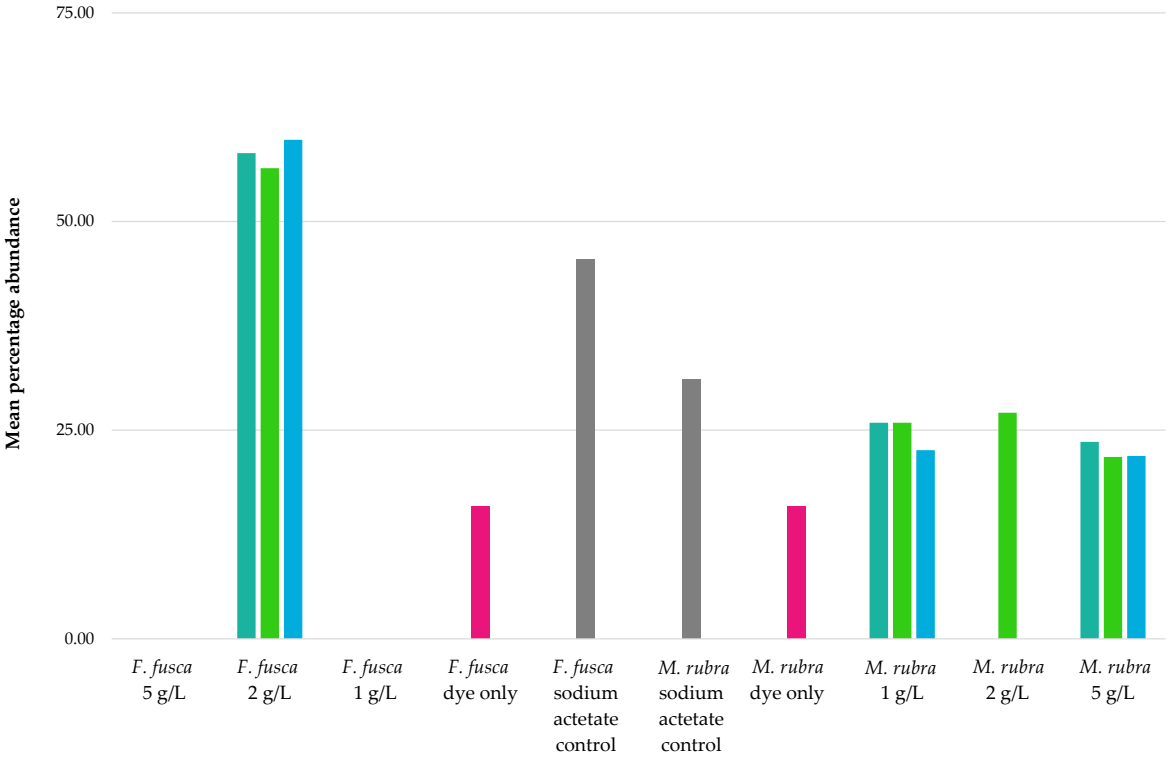


Figure 7.33: Bar chart showing a summary of the mean percentage abundances for each repeat and each of the tested groups for the dye rhodamine B.

ration is seen for *F. fusca*, this reflects the higher levels of fluorescence seen for this species. There is no real way of knowing why these trends are observed and the theory of biological stores has been discussed in great detail in previous chapters.

7.4 Conclusions

The work presented so far in this thesis has been formed of feeding experiments which involve diets chemically labelled with deuterium, or more commonly, ^{13}C atoms. The successful incorporation of these labelled substrates results in the detection of cuticular hydrocarbons which, due to the presence of the labels, have a slightly greater mass. So far the precursors used within this work have included acetate and propionate molecules, amino acids, and more complex unsaturated fatty acids. Throughout, differences have been noted, either between substrates or more usually between individuals. One theory for these differences has been related to the amount of food consumed; the greater the amount of labelled substrate consumed, the greater the amount of labelled substrate incorporated into the CHCs. This experiment was therefore designed to test this relationship; by adding fluorescent dyes to the labelled substrate diets it was hoped that the dyes could be used as a way of tracking the amount of food consumed and that this could be related to the amount of incorporation seen. In order to test this relationship two different dyes, at three different concentrations were tested against two species each. The fluorescence was measured from the resulting samples using a fluorescence imaging technique and the amount of incorporation was measured using the usual technique of GC-MS. Once all data analysis was performed each sample was ranked within its group based firstly on its brightness and then on the amount of incorporation seen. Scatter graphs were plotted showing the assigned ranks for each parameter in order to judge if there was correlation observed and Spearman's Rho (r_s) was also calculated.

For all of the tested groups there was no measureable correlation observed. This was somewhat surprising, however when looking at the raw data a lot of the values were very similar, meaning that some of the rank orders were fairly meaningless. This effect could have been reduced by stating the mean percentage abundances and brightnesses to just whole numbers, this would have led to large numbers of tied ranks and may have picked out some correlation between samples. However the most surprising observation

and the most important was that when analysing the data and ranking the samples manually, there were some samples that showed outliers; these samples may have been particularly bright, or showed particularly extreme amounts of incorporation. If the given theory was true, that food consumption and incorporation are linked, then it would at least be expected to have seen correlation between the extreme edges of the ranges. The species that showed the most difference in terms of the percentage incorporation values was *F. fusca*. For example the third repeat for the 2 g/L of rhodamine B showed a range of percentage abundances from 48.1% to 74.9%, for this type of data this is a huge range of values, looking at the raw data for this sheet in Appendix E.35 the differences can be easily seen between sample 3 and 5. This is the simplest way to test the theory that incorporation and consumption are linked; sample 3, with the lowest incorporation should show the dimmest fluorescence and vice versa for sample 5. This is not the case though, in fact the opposite argument was more appropriate for this set of data; sample 3 was one of the brightest, whilst sample 5 was one of the dimmest. However this assessment of relative brightness is fairly meaningless; the brightest and dimmest samples being separated by just 4.26. This highlights the main issue with this experiment; there was not enough variation shown by the samples, either in terms of incorporation, or in terms of brightness. If like the third repeat mentioned above, there was variation in percentage abundance then there was no variation in the mean brightness, and vice versa.

It may be argued that a more sensitive technique for measuring fluorescence would have indicated more variation. There is no doubt that whilst the photographic technique used to measure fluorescence was not as sensitive as other more sophisticated techniques, it was able to differentiate between brighter and dimmer samples, which was the main requirement when trying to find an alternative technique. It appears that there was a level of maximum brightness that was reached for the larger species of *F. fusca* and *F. lemani*, as mentioned this may be related to body volume and as such a weaker dye concentration may have been more appropriate for this species. This

may have led to more variation between samples, and a better relationship between the tested variables. It does seem that *F. fusca* is a good species to use for analysis of this type; the incorporation was highly variable compared to the values seen for *F. lemani* and *M. rubra*.

It also appears that the amount of possible fluorescence is directly affected by the size of the body, in particular the body volume. *M. rubra* consistently showed the smallest amounts of fluorescence, whilst the larger *Formica* species showed far greater amounts of fluorescence. It is clear that when designing an experiment of this type there is more to consider than just dyes and substrates. The size of the ant used is important, it must be large enough to be able to bio-accumulate the fluorescent dye and yet also show good levels of isoptopic incorporation. *F. fusca* provided an excellent study species in terms of these criteria, however a weaker dye concentration should have been tested.

Although the dye was used purely as a marker it is possible that it was influencing the amount of food eaten, a point which was overlooked when this experiment was designed. It is known that ants are able to see ultra-violet light [32] and hence there may have been an extra factor that caused more consumption of certain coloured, or more strongly coloured diets. However this is less of an issue as the key requirement to test is the relationship within the group and not between groups. This may however explain why different amounts of incorporation were seen for different dyes within the same species, or between the sodium [$^{13}C_2$]acetate controls and those containing dye. Again though, this is an argument based on a link between the amount of food consumed and the amount of incorporation seen.

Based on the findings of this experiment it is not possible to confirm, or deny that there is a relationship between the amount of food consumed and the amount of incorporation observed within the cuticular hydrocarbons. In order to further test this theory more experimentation should be undertaken. Firstly one species needs to be

chosen, as it seems that larger species are optimum as they have a better ability to bio-accumulate the dye. This species needs to be tested against a variety of dye concentrations to determine the optimum dye and concentration for that species. Whilst there may have been extra toxicity observed for rhodamine B, there was no conclusive trends observed, as such the use of this dye should not be ruled out. Finally once the ideal dye and concentration has been determined then this should be tested with a high number of repeats, and perhaps a lower number of samples per repeat, this will result in fewer ranks and perhaps a clearer trend.

References

[1] Bhatkar A, and Whitcomb WH, 'Artifical diet for rearing various species of ants,' (1970), *Florida Entomol.*, 53(4), 229-232

[2] Sudd JH, and Franks NR, *The Behavioural Ecology of Ants*, (Blackie, 1987)

[3] Walker GP, *Salivary Glands*, In 'Encyclopedia of Insects 2nd Edition' (Resh V and Cardé R), (Academic Press, 2009)

[4] Dillwith JW, Nelson JH, Pomonis JG, Nelson DR, and Blomquist GJ, 'A ^{13}C NMR study of methyl-branched hydrocarbon biosynthesis in the housefly,' (1982), *J. Biol. Chem.*, 257(19), 11305-11314

[5] Gu X, Quilici D, Juarez P, Blomquist GJ, and Schal C, 'Biosynthesis of hydrocarbons and contact sex pheromone and their transport by Lipophorin in females of the German cockroach *Blattella germanica*,' (1995), *J. Insect Physiol.*, 41(3), 257-267

[6] Chase J, Jurenka RA, Schal C, Halarnka PP, and Blomquist GJ, 'Biosynthesis of methyl-branched hydrocarbons of the German cockroach *Blattella germanica* (L.) (Orthoptera Blattellidae),' (1990), *Insect Biochem.*, 20(2), 149-156

[7] Young HP, and Schal C, 'Cuticular hydrocarbon synthesis in relation to feeding and developmental stage in nymphs of *Blattella germanica* (Dictyoptera: Blattellidae),' (1997), *Ann. Entomol. Soc. Am.*, 90(5), 655-663

[8] Campbell MK, and Farrell SO, *Biochemistry: 8th Edition*, (Cengage Learning, 2015)

[9] Atkins P, and De Paula J, *Elements of Physical Chemistry, 4th Edition*, (Oxford University Press, 2005)

[10] Rouessac F, and Rouessac A, *Chemical Analysis Modern Instrumentation Methods and Techniques* (John Wiley and Sons Inc., 2000)

[11] Langford A, Dean J, Reed R, Holmes D, Weyers J, and Jones A, *Practical Skills in Forensic Science*, (Pearson Education Limited, 2005)

[12] Christie RM, *Fluorescent Dyes*, In 'Handbook of Textile and Industrial Dyeing: Volume 1; Principles, Processes and Types of Dyes,' (Clark M) (Woodhead Publishing, 2011)

[13] Hou JT, Ren WX, Li K, Seo J, Sharma A, Yu XQ, and Kim JS, 'Fluorescent bioimaging of pH: from design to applications,' (2017), *Chem. Soc. Rev.*, 46, 2076-2090

[14] Dramićanin T, and Dramićanin M, *Using Fluorescence Spectroscopy to Diagnose Breast Cancer*, In 'Applications of Molecular Spectroscopy to Current Research in the Chemical and Biological Sciences' (Stauffer M) (Intech Open, 2016)

[15] Kallmeyer J, 'Contamination Control for Scientific Drilling Operations,' (2017), *Advances in Applied Microbiology*, 61-91

[16] Hicks JD, and Matthaei E, 'Fluorescence in Histology,' (1955), *The Journal of Pathology and Bacteriology*, 70(1)

[17] Prete C, Galmiche L, Quenum-Possy-Berry FG, Allain C, Thiburce N, and Colard T, 'LumicyanoTM: A new fluorescent cyanoacrylate for a one-step luminescent latent fingermark development,' (2013), *Forensic Science International*, 233, 104-122

[18] Sodhi G, Kaur J, and Garg RK, 'Fingerprint Powder Formulations Based on Organic, Fluorescent Dyes,' (2004), *Journal of Forensic Identification*, 54(1), 4-8

[19] Hagler JR, and Jackson CG, 'Methods for Marking Insects: Current Techniques and future Prospects,' (2001) *Annu. Rev. Entomol.*, 46, 511-543

[20] Greenwald E, Segre E, and Feinerman O, 'Ant trophallactic networks: simultaneous measurement of interaction patterns and food dissemination,'(2015), *Scientific Reports*, 5(12496)

[21] Gayahan GG, and Tschinkel WR, 'Fire Ants, *Solenopsis invicta*, Dry and Store Insect Pieces for Later Use,' (2008), *J. Insect Sci.*, 8(39)

[22] Tschinkel W, 'Vertical organization of the division of labor within nests of the Florida harvester ant, *Pogonomyrmex badius*,' (2017), *PLoS One*, 12(11)

[23] Strong Jr WR, Nelson DR, Bruce Barry B, and Murphy RD, '*Population dynamics of white sharks in Spencer Gulf, South Australia'*, In 'The Biology of *Carcharodon carcharias*,' (Klimley AP, and Ainley DG) (Elsevier, 1996)

[24] Slate D, Algeo TP, Nelson KM, Chipman RB, Donovon D, Blanton JD, Niezgoda M, and Rupprecht CE, 'Oral rabies vaccination in North America: opportunities, complexities and challenges,' (2009), *PLOS Negl. Trop. Dis.*, 3(12)

[25] Johnson BJ, Micthell SN, Paton CJ, Stevenson JS, Staunton KM, Snoad N, Beebe N, White BJ and Ritchie SA, 'Use of rhodamine B to mark the body and seminal fluid of male *Aedes aegypti* for mark-release-recapture experiments and estimating efficacy of sterile male releases,' (2017), *PLOS Negl. Trop. Dis.*, 11(9)

[26] Rowiński PM, and Chrzanowski MM, 'Influence of selected fluorescent dyes on small aquatic organisms,' (2011), *Acta Geophysica*, 59(1)

[27] Schramm S, and Weiß D, 'Fluorescent heterocycles: Recent trends and new developments,' (2019), *Advances in Heterocyclic Chemistry*, 128

[28] Sigma-Aldrich product catalogue at: https://www.sigmaaldrich.com/catalog/product/sigma/83689?lang=en&region=GB (accessed April 2019)

[29] Bumrah GS, 'Cyanoacrylate fuming method for detection of latent fingermarks: a review,' (2017), *Egypt J. Forensic Sci.*, 7(1), 4

[30] BVDA Forensic Supplies at: http://www.bvda.com/en/basic-yellow-40 (accessed July 2019)

[31] Lakowicz J,*Principles of Fluorescence Spectroscopy, 3rd Edition*, (Springer, 2006)

[32] Knight K, 'Ants prefer UV shades,' (2017), *J. Exp. Biol.*, 220,

Chapter 8

Conclusions & Future Work

8.1 Conclusions

8.1.1 Overview

The overall aim of this project was to use isotopically labelled substrates in feeding experiments to see if ants used the substrates to biosynthesise cuticular hydrocarbons. The results from each substrate and species could then be used to aid elucidation of the biosynthetic pathways, to see if there were apparent differences between published biosynthetic routes, determined from other species, and our studied ant species. All techniques and methods were based on those established from the literature or previous work.

8.1.2 Labelled substrate feeding experiments: acetates and propionates

The first of the experimental chapters focussed on the use of multiple species and simple labelled substrates based on acetate and propionate molecules. There were three individual experiments that made up this results chapter, and all three were carried out to test the experimental design, species suitability, and chemical and statistical analysis methods. It was the results of this experiment that showed that not all of the chosen studied species were suitable for this kind of work. Some species seemed to be less tolerant of the experimental conditions whilst some had chemical profiles which proved hard to analyse due to low abundances of cuticular hydrocarbons. It also be-

came apparent from these experiments that future work should be scaled up to include bigger sample sizes, more repeats and control populations. The results showed that all three of the initial study species *Formica lugubris*, *Formica lemani* and *Myrmica sabuleti* showed incorporation of the labelled acetate substrates sodium [1-^{13}C]acetate and sodium [2-^{13}C]acetate. This incorporation was found in all three classes of compound; alkenes, alkanes and methyl-branched hydrocarbons. However some issues with low control numbers for *Formica lugubris* means that this data is less reliable. It was found however that there were inter-species differences in the amount of incorporation shown. Although the reasons are unknown this could be down to food preference, or the nature of the biosynthetic process, for example a *de novo* synthesis will result in much greater incorporation than the modification of an existing long chain fatty acid. It was found that for this experiment the position of the label made little difference to the final amount of incorporation.

The results of the preliminary propionate experiment again showed much lower quality of results for *Formica lugubris*, however the results for *Formica lemani* and *Myrmica sabuleti* were much more insightful. The results for *F. lemani* showed clear incorporation of sodium [1-^{13}C]propionate into the methyl-branched hydrocarbons but very little incorporation of propionic-2,2-d_2 acid-d, with no clearly discernible preference for incorporation into a specific compound type. On the other hand the results for *Myrmica sabuleti* showed incorporation of both sodium [1-^{13}C]propionate and propionic-2,2-d_2 acid-d into the methyl-branched hydrocarbons. Like *F. lemani* this species also showed seemingly random incorporation into other compound types, however there was no pattern and this was attributed to the further metabolic breakdown of the propionate into acetate. The incorporation of propionic-2,2-d_2 acid-d into the methyl-branched hydrocarbons was a very interesting result as previously assumed biosynthetic pathways, based on literature, suggested that incorporation would not be seen. This might mean that for this species the biosynthetic pathway is slightly different to that previously elucidated from other species, or it could be due to some other unknown reason.

The final part of this chapter was an expansion of the propionate experiment using, as before, propionic-2,2-d_2 acid-d and *Myrmica sabuleti*, alongside a new species; *Myrmica scabrinodis*. This experiment was improved by increasing the sample sizes, number of repeats and the addition of controls. The results of this experiment were less clear. Although some incorporation was observed the clear results from the previous experiment involving *Myrmica sabuleti* and propionic-2,2-d_2 acid-d were not seen again. There was some incorporation seen for *Myrmica scabrinodis*, however whilst there was a definite bias shown towards incorporation of methyl-branched hydrocarbons, again this was not a totally conclusive result with statistical significance not observed for each methyl-branched compound at both studied M+n levels. Overall the results of these experiments were extremely useful as they provided a basis for future experiments, tested the methods and analysis, and showed that some species were better suited than others. More importantly the results served as a reminder that no assumptions, based on literature findings with other species, should be made for our ant species.

8.1.3 Labelled substrate feeding experiments: amino acids

The second series of experiments carried out focussed on the use of amino acid based substrates. The choice was largely driven by commercial availability and budgetary constraints. Initially work focussed on the use of the deuterated substrates DL-valine d_8 and L-leucine 5,5,5-d_3 as these were much cheaper to obtain than those labelled with ^{13}C. The specific amino acid choice was firstly based on commercial availability but also lead by existing research. It is known that valine, isoleucine and methionine can be metabolised to form propionates, therefore valine was chosen as an amino acid from this group, whilst leucine was chosen because it was not one of these three. The two species used were *Formica lemani* and *Myrmica sabuleti*. Somewhat surprisingly the results for *Formica lemani* showed limited incorporation, even for DL-valine d_8, which literature suggested should have shown incorporation. The results for *Myrmica sabuleti* were more promising with incorporation seen for the methyl-branched hydrocarbons

and DL-valine d_8. Whilst these results almost followed what was expected, there were also problems with the quality of the data and it was felt that this and the potential labile nature of the deuterium label may have been adversely affecting the quality of the result and possibly hiding any weak incorporation.

Towards the end of the project it was therefore decided to purchase additional ^{13}C labelled substrates and try the experiments using these, in the hope that they would provide clearer results. The data from these experiments was extremely interesting and showed large amounts of very clear incorporation, although unfortunately little data was obtainable from the experiment with *Formica lemani* and L-valine $^{13}C_5$,^{15}N. However from the results of the other experiments involving ^{13}C labelled amino acids it was possible to conclude that both species are able to break down both amino acids into smaller molecules, likely acetyl-CoA molecules and subsequently use them during the biosynthesis of all hydrocarbons. The results also showed more subtle differences in the incorporation depending on the species and compound type suggesting a complex series of possible metabolic steps. However the most useful finding was that leucine, previously thought to play only a minor role in hydrocarbon biosynthesis could, in fact, have a much more important function than originally realised.

8.1.4 Labelled substrate feeding experiments: fatty acids

The final substrate study involved the bespoke synthesis of labelled unsaturated fatty acids with deuterium labels across the double bond. In total 12 fatty acids were synthesised with different chain lengths and double bond positions. The species chosen for this study was *Myrmica sabuleti* due to its proven reliability. The results for each synthesised fatty acid were different, however there were a few substrates that showed incorporation for pentacosane and $I^{(M+2)}$. This could be due to a secondary mechanism which involves the hydrogenation of the unsaturated fatty acid into the saturated equivalent which is then converted into an alkane. However this theory does not en-

tirely explain why this is seen only for pentacosane. The other reason is to do with the control procedure used for this study. The inclusion of specific substrate control would have meant that two batches of the fatty acid would have to be synthesised, a labelled version and an unlabelled version. As this would have resulted in twice the work and twice the cost, the decision was made not to do this. Instead the control values were taken from 20 previously obtained *Myrmica sabuleti* profiles. These control profiles were the same for each tested substrate, and as a result, if this control data value for pentacosane was lower than that theoretically expected then increased statistical significance for the substrate data would be more likely. Comparison of the experimentally derived control value for pentacosane compared to a theoretically calculated value showed that the calculated value was indeed greater suggesting that this could be the reason for the statistical significance seen for pentacosane.

It was also seen that statistically significant levels of incorporation were seen for several compounds and $I^{(M+1)}$ only. This was attributed to the β-oxidation of the fatty acid to ultimately yield a molecule of singularly labelled acetyl-CoA, however this could also be the result of some other process.

The most significant finding from this study was that seen for octadec-11-enoic acid-11,12-d_2. The results from this substrate showed that when fed this substrate *Myrmica sabuleti* ants started to produce a Z7 positional isomer, one that is not normally found for this species. Interestingly this positional isomer is found within the chemical profile of the closely related *Myrmica scabrinodis*. Therefore an extremely tentative possibility could be that these two species share biosynthetic pathways, but only certain pathways are active within the species to keep them chemically distinct with species specific chemical cues. However the reason why this effect was seen for this compound is unknown. It should also be remembered that all substrates used in this study utilised deuterium labels which are known to be more labile and it is possible that some trends are masked by their usage.

8.1.5 A study into the relationship between substrate incorporation and amount of food consumed

So far, the work presented in this thesis had consisted of simple feeding experiments involving chemically labelled diets. These experiments were designed to help indicate what precursors could be used in the biosynthesis of cuticular hydrocarbons. Throughout this thesis, differences in incorporation have been observed, either between substrates, species or even on an individual basis. Of the theories for these differences has been related to the amount of food consumed; the more labelled substrate consumed, the greater the incorporation. This experiment was therefore designed to test this relationship.

Several concentrations of two different dyes and three species were tested, but despite testing many different combinations, none was found that showed any direct link between the amount of food eaten and the incorporation shown. Possible reasons for this were explored within the specific chapter. As previously seen there were however some individuals that showed much higher incorporation of the isotopic labels than others, but when combined with the brightness values there was no correlation between them. If there truly existed a direct link between the two then it would be expected that at the very least this would be evident when looking at the extreme outlying values, however this was not the case. The maximum and minimum values for mean brightness and abundance were not consistently attributed to the same samples within a group. On this basis it cannot be said that there is any link between the amount of food eaten and the amount of incorporation shown

Despite this lack of direct relationship there were a few interesting observations. Firstly it seemed that each ant had a maximum brightness that was achievable, a saturation of dye within its body. This led to some brightness values that were very similar and therefore difficult to meaningfully rank. This may have been due to the body size of the

ant and hence it seems that smaller ants were less suited to this kind of work. If this work was to be repeated, larger ant species and a greater range of dye concentrations would be a useful expansion. It was also expected that the mean incorporation would be similar for the experimental diets compared to the sodium [$^{13}C_2$]acetate control diets. However this was not the case, it was postulated that the presence of the dye may have some effect on the desire of the ants to consume the food, but again no conclusive link was determined.

The work presented in this chapter was useful in that it again suggests that to assume that there is simplistic link between factors is naive. Instead it must be assumed that due to the complexities of biological organisms, such as ants, there is no simplistic relationship and instead the amount of incorporation is dependant upon many complex factors.

8.2 Future Work

Overall this project has shown some very interesting results and unexpected findings. The work presented here has suggested that previously accepted biosynthetic routes may well be different for our studied species and that the same pathways cannot be assumed for all insect species. However it has also highlighted the difficulties with work of this type. The main issue is the availability of the substrates. There is no doubt that the usage of deuterium labels is limited in terms of their success; far clearer results were seen when labels were carbon based. Future work should therefore focus on the use of carbon based labels and possible bespoke synthesis of substrates so that a wider variety can be tested on many different species. The use of ^{13}C labels also ultimately means that ^{13}C NMR analysis of the hydrocarbons can be performed to ascertain the resulting position of the label. This may provide extra information as to the biosynthetic routes used.

The study on amino acids revealed some interesting results and should be expanded to include more ^{13}C labelled amino acids, both those expected to give incorporation i.e. methionine and isoleucine and those not. It is also worth repeating these experiments as the repeatability and reproducibility of the results has not been explored. It is entirely possible that an entire project's worth of data could be collected from just one type of substrate, be it amino, or fatty acids. In addition it goes without saying that the there is huge potential for the range of fatty acids to be expanded upon however these should be synthesised with ^{13}C labels.

There is also work which focusses on issues other than the species and the substrate but which could ultimately be just as important. This study used fragments of wild colonies, whose age and status were not known. The differences in populations and samples used likely had some effect on subsequent results. The possibility of this should therefore also be explored by rearing ants through subsequent generations to ensure

that the individuals used for experiments are as consistent as possible, i.e. with a known approximate age and background. The results from lab reared ants could then be compared to wild ants to see if there is a difference in the incorporation seen as it is believed that natural stores of biologically important molecules could be an important factor. In addition there are many other factors which may affect the rate of hydrocarbon production, including worker role, temperature and humidity. For example it would be interesting to see whether ants kept in a low temperature versus a high temperature show differences in the levels of incorporation as temperature was not a factor controlled within any of these studies.

The results of this project show that there is enormous potential in biosynthetic pathway elucidation using simple feeding experiments. There were many external factors not controlled in the studies presented here so their impact is not known. There are many species of ants with very different chemical profiles which may show completely different results with other substrates. However what is clear is that all studies need to be as robust as possible with suitable sample sizes, repeats and controls. All experiments must also utilise a wide variety of suitable substrates with ^{13}C labels, and the use of ^{13}C NMR should be considered in order to gain a better insight into the results and biosynthetic processes.

References

[1] Arrese EL, and Soulages JL, 'Insect fat body: Energy, metabolism, and regulation,' (2010), *Annu. Rev. Entomol.*, 55, 207-225

[2] Anton MM, *Composition and Structure of Hen Egg Yolk*, In 'Bioactive Egg Compounds' (Huopalahti R, López-Fandiño R, Anton M and Schade R) (Springer Berlin Heidelberg, 2007)

[3] Bitman J, *Status Report on the Alteration of Fatty Acids and Sterol Composition in Lipids in Meat, Milk and Eggs*, In 'Fat Content and Composition of Animal Products: Proceedings of a Symposium' (National Research Council) (National Academy of Sciences, 1976)

[4] Food Standards Agency, *McCance and Widdowson's The Composition of Foods 6th summary ed.*, (The Royal Society of Chemistry, 2002)

Appendix A

Typical chromatograms for each study species with accompanying compound identification tables

Figure A.1: Chromatogram showing the chemical profile of *Formica lugubris*
Table A.1: The main cuticular hydrocarbons present on *Formica lugubris*

Figure A.2: Chromatogram showing the chemical profile of *Formica lemani*
Table A.2: The main cuticular hydrocarbons present on *Formica lemani*

Figure A.3: Chromatogram showing the chemical profile of *Myrmica scabrinodis*
Table A.3: The main cuticular hydrocarbons present on *Myrmica scabrinodis*

Figure A.4: Chromatogram showing the chemical profile of *Myrmica sabuleti*
Table A.4: The main cuticular hydrocarbons present on *Myrmica sabuleti*

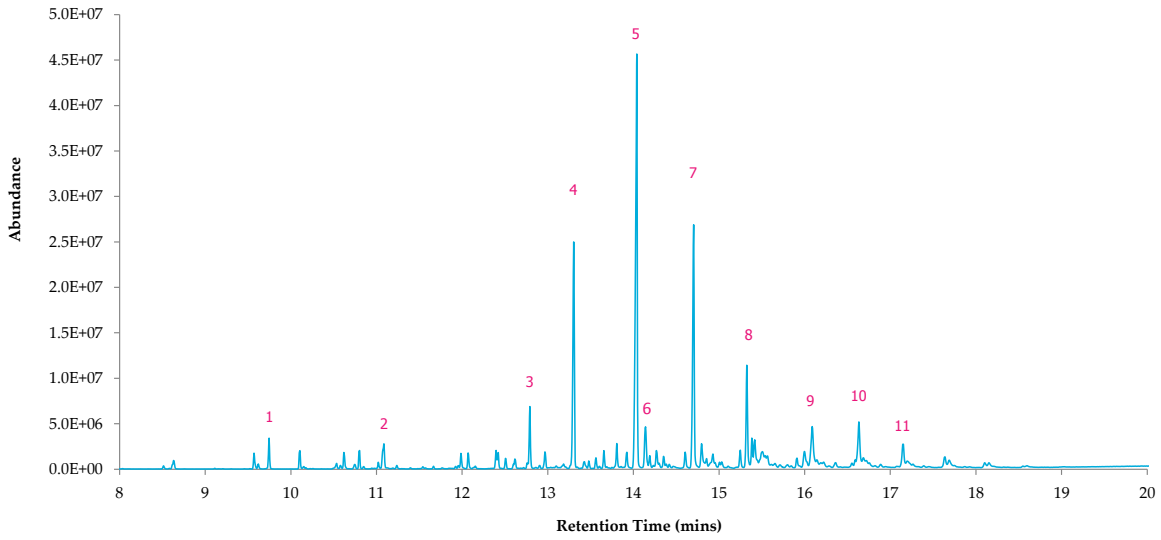


Figure A.1: Typical chemical profile of a *Formica lugubris* ant.

Table A.1: The main cuticular hydrocarbons present within the chemical profile of *Formica lugubris*. Note that in the table below 'x' is used to abbreviate the second position of the dimethyl compounds.

Compound number	Retention time (mins)	Compound identity
1	9.746	Heptadecane
2	11.088	Hexadecadiene
3	12.791	Unknown
4	13.303	Pentacosane
5	14.041	Heptacosane
6	14.142	9-, 11-, & 13-methylheptacosane mix
7	14.704	Nonacosane
8	15.324	Hentriacontane
9	16.088	11,x-, 13,x-, & 15,x-dimethyl tritriacontane mix
10	16.633	11,x-, 13,x-, 15,x-, & 17,x-dimethyl pentatriacontane mix
11	17.153	11,x-, 13,x-, 15,x-, 17,x-, & 19,x-dimethyl heptatriacontane mix

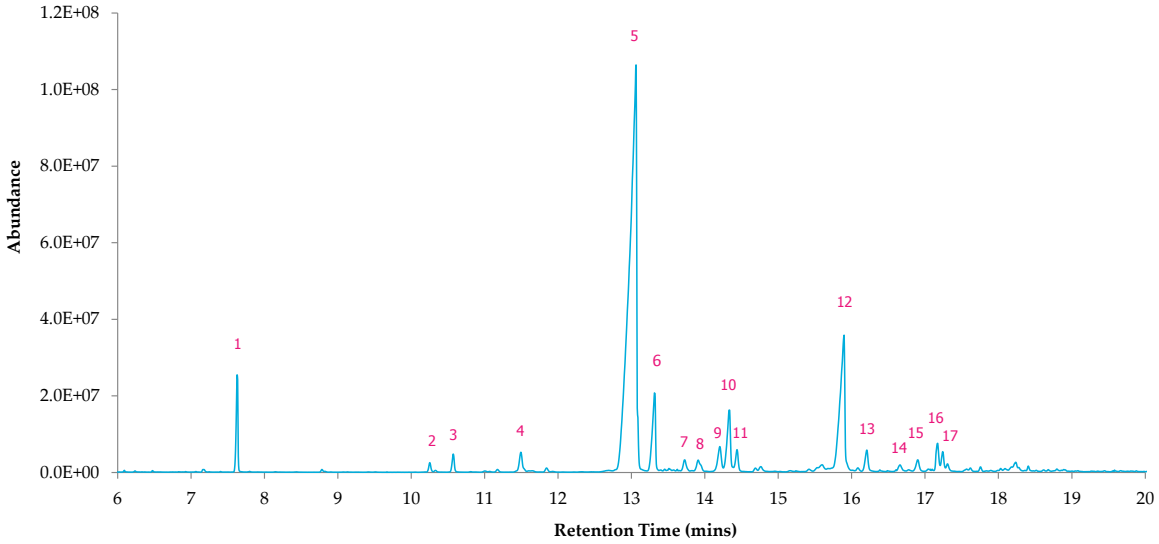


Figure A.2: Typical chemical profile of a *Formica lemani* ant.

Table A.2: The main cuticular hydrocarbons present within the chemical profile of *Formica lemani*.

Compound number	Retention time (mins)	Compound identity
1	7.618	Eicosane
2	10.252	Tricosene
3	10.571	Tricosane
4	11.494	3-methyltricosane
5	13.063	Pentacosene
6	13.306	Pentacosane
7	13.725	11, & 13-methylpentacosane
8	13.910	Unsaturated fatty alcohol
9	14.204	Unsaturated fatty alcohol (likely C25:1)
10	14.329	3-methylpentacosane
11	14.438	5,15-dimethylpentacosane
12	15.898	Heptacosene
13	16.208	Heptacosane
14	16.661	11-, & 13-methylheptacosane
15	16.905	Unknown
16	17.240	3-methylheptacosane
17	16.905	5,15-dimethylheptacosane

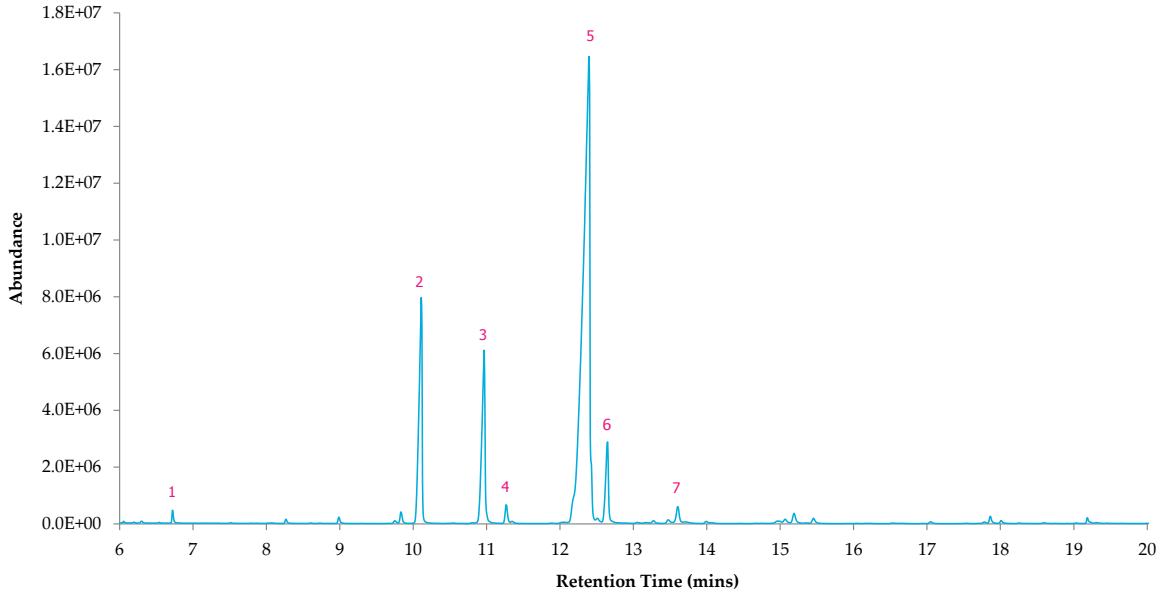


Figure A.3: Typical chemical profile of a *Myrmica scabrinodis* ant.

Table A.3: The main cuticular hydrocarbons present within the chemical profile of *Myrmica scabrinodis*.

Compound number	Retention time (mins)	Compound identity
1	6.720	1-Bromo-8-tetrahydropyranyloxyoctane
2	10.110	Tricosane
3	10.968	3-methyltricosane
4	11.265	Tetracosane
5	12.395	Pentacosene
6	12.650	Pentacosane
7	13.602	3-methylpentacosane

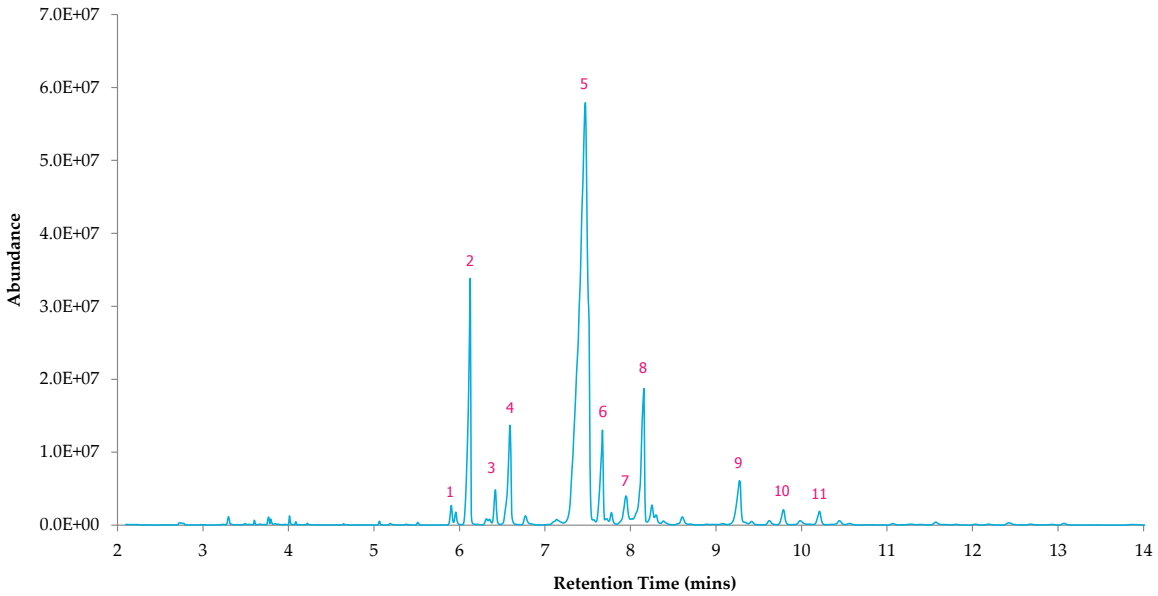


Figure A.4: Typical chemical profile of a *Myrmica sabuleti* ant.

Table A.4: The main cuticular hydrocarbons present within the chemical profile of *Myrmica sabuleti*.

Compound number	Retention time (mins)	Compound identity
1	5.906	Tricosene
2	6.127	Tricosane
3	6.422	5-methyltricosane
4	6.590	3-methyltricosane
5	7.474	Pentacosene
6	7.674	Pentacosane
7	7.948	11-, & 13-methylpentacosane
8	8.158	5-methylpentacosane
9	9.273	Heptacosene
10	9.789	Unknown
11	10.210	5-methylheptacosane

Appendix B

Excel data sheets of the raw data for Chapter 4

The following pages show the raw data sheets from the acetate and propionate substrate experiments. The cells are colour coded according to the percentage abundance calculated from the raw mass spectra data, see Section 3.4.2.1. In some cases only partial data is shown due to the experimental success. For the later experiments the data extends across several pages.

Preliminary sodium acetate experiment

Figure B.1: Raw substrate data for *Formica lugubris* and sodium [1-^{13}C]acetate
Figure B.2: Raw control data for *Formica lugubris* and sodium [2-^{13}C]acetate
Figure B.3: Raw substrate data for *Formica lugubris* and sodium [2-^{13}C]acetate
Figure B.4: Raw control data for *Formica lemani* and sodium [1-^{13}C]acetate
Figure B.5: Raw substrate data for *Formica lemani* and sodium [1-^{13}C]acetate
Figure B.6: Raw control data for *Formica lemani* and sodium [2-^{13}C]acetate
Figure B.7: Raw substrate data for *Formica lemani* and sodium [2-^{13}C]acetate
Figure B.8: Raw control data for *Myrmica sabuleti* and sodium [1-^{13}C]acetate
Figure B.9: Raw substrate data for *Myrmica sabuleti* and sodium [1-^{13}C]acetate
Figure B.10: Raw control data for *Myrmica sabuleti* and sodium [2-^{13}C]acetate
Figure B.11: Raw substrate data for *Myrmica sabuleti* and sodium [2-^{13}C]acetate

Preliminary propionate experiment

Figure B.12: Raw control data for *Formica lugubris* and propionic acid
Figure B.13: Raw substrate data for *Formica lugubris* and propionic-2,2-d_2 acid-d
Figure B.14: Raw control data for *Formica lemani* and propionic acid
Figure B.15: Raw substrate data for *Formica lemani* and sodium [1-^{13}C]propionate
Figure B.16: Raw substrate data for *Formica lemani* and propionic-2,2-d_2 acid-d
Figure B.17: Raw control data for *Myrmica sabuleti* and propionic acid
Figure B.18: Raw substrate data for *Myrmica sabuleti* and sodium [1-^{13}C]propionate
Figure B.19: Raw substrate data for *Myrmica sabuleti* and propionic-2,2-d_2 acid-d

Propionate experiment

Figures B.20-B.22: Raw negative control data for *Myrmica sabuleti*
Figures B.23-B.24: Raw positive control data for *Myrmica sabuleti*
Figures B.25-B.28: Raw substrate data for *Myrmica sabuleti*
Figures B.29-B.31: Raw negative control data for *Myrmica scabrinodis*
Figures B.32-B.34: Raw positive control data for *Myrmica scabrinodis*
Figures B.35-B.36: Raw substrate data for *Myrmica scabrinodis*

Sodium [1-^{13}C]acetate		*Formica lugubris*									
		Sub 1	Sub 2	Sub 3	Sub 4	Sub 5	Sub 6	Sub 7	Sub 8	Sub 9	Sub 10
Abundance of M	Pentacosane	4298	2862	12917	1473	5155	4819	9024	2888	4278	
	Heptacosane	8394	20568	12371	5068	15179	6602	11936	8649	2711	906
	Nonacosane	9246	11883	11883	11760	7856	9783	14354	7221	3506	2742
	11, 13, 15 Me C29	37008	26592	19896	15549	32936	32936	40832	27504	2849	3909
	Hentriacontene	27304	30080	21760	13377	18072	35176	23872	16784	5026	466
Abundance of M+1	Pentacosane	4219	5254	9981	3682	5060	7928	10359	4193	2410	
	Heptacosane	7903	17928	14368	7653	17472	13890	16640	12744	2593	706
	Nonacosane	17232	24736	24736	11354	8911	20104	20112	14054	1932	985
	11, 13, 15 Me C29	54840	51600	29464	28192	49328	49328	47592	60008	5974	6278
	Hentriacontene	60208	69032	35632	25256	33632	76248	46040	38440	5104	1740
Abundance of M+2	Pentacosane	6878	7368	9710	2658	4219	8160	7008	6724	1805	
	Heptacosane	11592	8353	11870	8051	13481	14841	13147	10265	1018	993
	Nonacosane	19096	27656	27656	12825	7480	24504	16447	13174	3191	1355
	11, 13, 15 Me C29	42872	33520	21152	12716	32536	32536	36352	36648	3822	3312
	Hentriacontene	76992	88648	30296	29984	34752	98560	47216	45216	10595	1723
Percentage of M+1	Pentacosane	98.2	183.6	77.3	250.0	98.2	164.5	114.8	145.2	56.3	
	Heptacosane	94.2	87.2	116.1	151.0	115.1	210.4	139.4	147.3	95.6	77.9
	Nonacosane	186.4	208.2	208.2	96.5	113.4	205.5	140.1	194.6	55.1	35.9
	11, 13, 15 Me C29	148.2	194.0	148.1	181.3	149.8	149.8	116.6	218.2	209.7	160.6
	Hentriacontene	220.5	229.5	163.8	188.8	186.1	216.8	192.9	229.0	101.6	373.4
Percentage of M+2	Pentacosane	160.0	257.4	75.2	180.4	81.8	169.3	77.7	232.8	42.2	
	Heptacosane	138.1	40.6	96.0	158.9	88.8	224.8	110.1	118.7	37.6	109.6
	Nonacosane	206.5	232.7	232.7	109.1	95.2	250.5	114.6	182.4	91.0	49.4
	11, 13, 15 Me C29	115.8	126.1	106.3	81.8	98.8	98.8	89.0	133.2	134.2	84.7
	Hentriacontene	282.0	294.7	139.2	224.1	192.3	280.2	197.8	269.4	210.8	369.7

0
1-20
20-40
40-60
60-80
80-100
100+

Figure B.1: Data sheet showing the raw substrate data for the preliminary sodium [1-^{13}C]acetate experiment for *Formica lugubris*. Note that for this experiment there was insufficient data for the control group, therefore it is not shown here.

Sodium [2-^{13}C]acetate		*Formica lugubris*									
		Con 1	Con 2	Con 3	Con 4	Con 5	Con 6	Con 7	Con 8	Con 9	Con 10
Abundance of M	Pentacosane	7609	5447	4083	5877	15122	14108	4894	5668	6373	4203
	Heptacosane	9969	11221	16400	9094	18480	22200	13434	15854	10348	8316
	Nonacosane	9053	9838	25456	15965	28024	19392	21280	19464	11030	4429
	11, 13, 15 Me C29	7515	7974	7844	11861	11837	35376		7064	9292	3377
	Hentriacontene	5191	5423	3212	8281	9847	17304		3971	4483	1730
Abundance of M+1	Pentacosane	961	1639	1401	1058	3206	4557	1212	1536	2109	1277
	Heptacosane	2946	3437	4155	2719	5042	7382	4505	3461	2582	2284
	Nonacosane	2697	3114	7884	6184	9112	4427	6575	5224	2587	1358
	11, 13, 15 Me C29	6641	5902	5819	8116	7904	18994	no data	4280	4985	1912
	Hentriacontene	2300	2528	1620	3633	3535	4565	no data	1201	2193	1022
Abundance of M+2	Pentacosane	516	0	0	289	623	698	339		654	341
	Heptacosane	453	535	692	406	442	1001	688	1052	780	728
	Nonacosane	359	680	1200	650	1352	860	1300	1099	667	157
	11, 13, 15 Me C29	767	1055	557	855	1137	3445		398	1066	205
	Hentriacontene	569	377	220	839	1248	1241			223	0
Percentage of M+1	Pentacosane	12.6	30.1	34.3	18.0	21.2	32.3	24.8	27.1	33.1	30.4
	Heptacosane	29.6	30.6	25.3	29.9	27.3	33.3	33.5	21.8	25.0	27.5
	Nonacosane	29.8	31.7	31.0	38.7	32.5	22.8	30.9	26.8	23.5	30.7
	11, 13, 15 Me C29	88.4	74.0	74.2	68.4	66.8	53.7		60.6	53.6	56.6
	Hentriacontene	44.3	46.6	50.4	43.9	35.9	26.4		30.2	48.9	59.1
Percentage of M+2	Pentacosane	6.8	0.0	0.0	4.9	4.1	4.9	6.9	0.0	10.3	8.1
	Heptacosane	4.5	4.8	4.2	4.5	2.4	4.5	5.1	6.6	7.5	8.8
	Nonacosane	4.0	6.9	4.7	4.1	4.8	4.4	6.1	5.6	6.0	3.5
	11, 13, 15 Me C29	10.2	13.2	7.1	7.2	9.6	9.7		5.6	11.5	6.1
	Hentriacontene	11.0	7.0	6.8	10.1	12.7	7.2		0.0	5.0	0.0

0
1-20
20-40
40-60
60-80
80-100
100+

Figure B.2: Data sheet showing the raw control data for the preliminary sodium [2-^{13}C]acetate experiment for *Formica lugubris*.

Sodium [2-^{13}C]acetate		*Formica lugubris*									
		Sub 1	Sub 2	Sub 3	Sub 4	Sub 5	Sub 6	Sub 7	Sub 8	Sub 9	Sub 10
Abundance of M	Pentacosane	3286	5950	3356	6793	3517	2144	5483	4497	3273	3422
	Heptacosane	4922	9270	4017	7031	6229	6473	5702	5378	6522	7395
	Nonacosane	5897	7984	7143	7988	7973	8291	9243	6013	5934	7059
	11, 13, 15 Me C29	1082	2862	2379	7403	11604	9278	3077	5317	4586	2073
	Hentriacontene	230	875	408	884	3445	2236	493	764	448	495
Abundance of M+1	Pentacosane	1359	3229	1782	1925	1270	1073	2036	1264	2703	1619
	Heptacosane	2501	5263	1805	2519	2312	3127	2635	2230	3140	2616
	Nonacosane	2246	5215	3052	3465	3597	4120	3738	2625	2787	3179
	11, 13, 15 Me C29	2051	4603	3739	8550	12937	11981	3602	6228	6054	3643
	Hentriacontene	276	709	329	750	2937	1424	577	1246	567	575
Abundance of M+2	Pentacosane	460	4404	1439	1471	1335	605	848	990	1128	421
	Heptacosane	1088	5677	1862	3139	1413	1394	2124	2719	2476	1231
	Nonacosane	1868	6270	2258	2121	2791	1239	1816	1878	1956	1094
	11, 13, 15 Me C29	2152	3810	2296	6427	11191	11450	2129	6590	3806	2811
	Hentriacontene	965	774	752	1483	5125	2226	546	1048	574	588
Percentage of M+1	Pentacosane	41.4	54.3	53.1	28.3	36.1	50.0	37.1	28.1	82.6	47.3
	Heptacosane	50.8	56.8	44.9	35.8	37.1	48.3	46.2	41.5	48.1	35.4
	Nonacosane	38.1	65.3	42.7	43.4	45.1	49.7	40.4	43.7	47.0	45.0
	11, 13, 15 Me C29	189.6	160.8	157.2	115.5	111.5	129.1	117.1	117.1	132.0	175.7
	Hentriacontene	120.0	81.0	80.6	84.8	85.3	63.7	117.0	163.1	126.6	116.2
Percentage of M+2	Pentacosane	14.0	74.0	42.9	21.7	38.0	28.2	15.5	22.0	34.5	12.3
	Heptacosane	22.1	61.2	46.4	44.6	22.7	21.5	37.3	50.6	38.0	16.6
	Nonacosane	31.7	78.5	31.6	26.6	35.0	14.9	19.6	31.2	33.0	15.5
	11, 13, 15 Me C29	198.9	133.1	96.5	86.8	96.4	123.4	69.2	123.9	83.0	135.6
	Hentriacontene	419.6	88.5	184.3	167.8	148.8	99.6	110.8	137.2	128.1	118.8

0
1-20
20-40
40-60
60-80
80-100
100+

Figure B.3: Data sheet showing the raw substrate data for the preliminary sodium [2-^{13}C]acetate experiment for *Formica lugubris*. Note that for this experiment there was insufficient data for the control group, therefore it is not shown here.

Sodium acetate (Control)		*Formica lemani*									
		Con 1	Con 2	Con 3	Con 4	Con 5	Con 6	Con 7	Con 8	Con 9	Con 10
Abundance of M	Pentacosene	1883648	1805824	1355264	1273344	2114048	1648640	1627648	2105856	2088448	1731072
	Pentacosane	121173	80704	86240	58848	156224	88872	91104	169984	161408	124608
	3 Me C25	988224	836288	622528	624576	1116160	785920	844096	1115136	1185280	851776
	Heptacosene	404418	465152	350592	369152	593984	456256	467328	609408	587520	520512
	Heptacosane	22896	18088	13574	13057	41280	18656	15121	46800	35944	26032
Abundance of M+1	Pentacosene	490496	461184	334848	337536	590912	412224	413824	534656	536064	455808
	Pentacosane	31047	23104	21024	16102	35752	25008	28744	53840	44200	37608
	3 Me C25	235072	219072	158848	145984	257536	193856	206528	299328	273664	204480
	Heptacosene	141775	137216	104712	104672	180416	132672	145344	162496	169984	155136
	Heptacosane	7260	3180	3483	4647	10358	5237	4366	14760	9579	9279
Abundance of M+2	Pentacosene	59960	56968	44256	50064	67784	48128	51640	63376	73680	57128
	Pentacosane	7238	2803	5570	4605	5644	3209	3472	4613	7278	3574
	3 Me C25	24506	27008	16872	16297	30896	23096	23208	43064	29472	29512
	Heptacosene	16319	21080	15543	15989	29776	15463	18928	22640	25984	22240
	Heptacosane	831	832	612	609	1250	709	275	1706	2884	1941
Percentage of M+1	Pentacosene	26.0	25.5	24.7	26.5	28.0	25.0	25.4	25.4	25.7	26.3
	Pentacosane	25.6	28.6	24.4	27.4	22.9	28.1	31.6	31.7	27.4	30.2
	3 Me C25	23.8	26.2	25.5	23.4	23.1	24.7	24.5	26.8	23.1	24.0
	Heptacosene	35.1	29.5	29.9	28.4	30.4	29.1	31.1	26.7	28.9	29.8
	Heptacosane	31.7	17.6	25.7	35.6	25.1	28.1	28.9	31.5	26.6	35.6
Percentage of M+2	Pentacosene	3.2	3.2	3.3	3.9	3.2	2.9	3.2	3.0	3.5	3.3
	Pentacosane	6.0	3.5	6.5	7.8	3.6	3.6	3.8	2.7	4.5	2.9
	3 Me C25	2.5	3.2	2.7	2.6	2.8	2.9	2.7	3.9	2.5	3.5
	Heptacosene	4.0	4.5	4.4	4.3	5.0	3.4	4.1	3.7	4.4	4.3
	Heptacosane	3.6	4.6	4.5	4.7	3.0	3.8	1.8	3.6	8.0	7.5

0
1-20
20-40
40-60
60-80
80-100
100+

Figure B.4: Data sheet showing the raw control data for the preliminary sodium [1-^{13}C]acetate experiment for *Formica lemani*.

Sodium [1-^{13}C]acetate		*Formica lemani*									
		Sub 1	Sub 2	Sub 3	Sub 4	Sub 5	Sub 6	Sub 7	Sub 8	Sub 9	Sub 10
Abundance of M	Pentacosene	2676224	1624576	2183168	2197504	2963456	3957760	1030016	1972736	2399232	2264576
	Pentacosane	243648	153216	202880	170624	194496	208768	66352	139712	144576	202048
	3 Me C25	1515520	870976	1154048	1178112	1608704	2212864	501504	1046464	1147904	1378304
	Heptacosene	690944	428096	585280	554624	787776	1001216	271936	543040	637056	619712
	Heptacosane	80736	56616	2197504	47320	43312	38768	23240	28928	30416	45456
Abundance of M+1	Pentacosene	825920	516928	659200	689408	875648	1166336	332544	596864	715904	718144
	Pentacosane	82272	56568	71728	64728	53064	73080	26168	47752	48304	66904
	3 Me C25	412736	258240	324096	323072	434752	591040	150080	295680	310848	377408
	Heptacosene	262272	173632	217408	227008	271040	372672	101184	194560	242240	223168
	Heptacosane	30512	19792	689408	12458	14287	13533	8508	7257	12167	12873
Abundance of M+2	Pentacosene	167168	124008	153536	155904	177472	241472	64880	124136	135616	143680
	Pentacosane	31400	25592	27216	13690	18000	18880	6541	6759	15392	23000
	3 Me C25	87088	56744	71904	71440	94136	124064	35240	75672	69024	76376
	Heptacosene	80864	69976	67720	65056	79448	119184	38784	51960	80008	79432
	Heptacosane	17912	8110	155904	4658	4544	8092	614	710	1704	9369
Percentage of M+1	Pentacosene	30.9	31.8	30.2	31.4	29.5	29.5	32.3	30.3	29.8	31.7
	Pentacosane	33.8	36.9	35.4	37.9	27.3	35.0	39.4	34.2	33.4	33.1
	3 Me C25	27.2	29.6	28.1	27.4	27.0	26.7	29.9	28.3	27.1	27.4
	Heptacosene	38.0	40.6	37.1	40.9	34.4	37.2	37.2	35.8	38.0	36.0
	Heptacosane	37.8	35.0	31.4	26.3	33.0	34.9	36.6	25.1	40.0	28.3
Percentage of M+2	Pentacosene	6.2	7.6	7.0	7.1	6.0	6.1	6.3	6.3	5.7	6.3
	Pentacosane	12.9	16.7	13.4	8.0	9.3	9.0	9.9	4.8	10.6	11.4
	3 Me C25	5.7	6.5	6.2	6.1	5.9	5.6	7.0	7.2	6.0	5.5
	Heptacosene	11.7	16.3	11.6	11.7	10.1	11.9	14.3	9.6	12.6	12.8
	Heptacosane	22.2	14.3	7.1	9.8	10.5	20.9	2.6	2.5	5.6	20.6

0
1-20
20-40
40-60
60-80
80-100
100+

Figure B.5: Data sheet showing the raw substrate data for the preliminary sodium [1-^{13}C]acetate experiment for *Formica lemani*.

Sodium [2-^{13}C]acetate		*Formica lemani*									
		Con 1	Con 2	Con 3	Con 4	Con 5	Con 6	Con 7	Con 8	Con 9	Con 10
Abundance of M	Pentacosene	168896	188928	161152	505984	71208	279616	136512	185408	143040	98432
	Pentacosane	54016	45656	32368	64432	37720	51152	34096	29224	46720	51576
	3 Me C25	111592	119336	115832	275328	30872	134208	68016	77752	75736	74760
	Heptacosene	43296	46392	58536	148864	16864	69056	30280	47048	36096	18456
	Heptacosane	10425	10279	8607	14579	9145	13330	8003	8457	13909	14681
Abundance of M+1	Pentacosene	47280	47360	42472	139776	19600	72280	38696	48264	38424	26576
	Pentacosane	15622	9449	8964	16432	10650	13480	8411	7069	14744	15846
	3 Me C25	28944	27928	30960	74192	8397	33312	17552	17992	20136	18896
	Heptacosene	12893	14271	15285	46728	3583	21680	11475	11822	9841	6285
	Heptacosane	3538	3564	3136	5283	2884	3330	2600	2433	4013	3733
Abundance of M+2	Pentacosene	5866	6978	4863	22048	2757	10845	4996	8275	5936	4447
	Pentacosane	2710	2205	1624	2349	2136	1460	1450	1147	2762	1694
	3 Me C25	4375	4658	3588	9496	1396	5217	1782	2934	3394	2326
	Heptacosene	1606	2413	3072	8443	702	3223	1982	2010	1489	1028
	Heptacosane	809	805	396	1070	719	658	680	502	770	1166
Percentage of M+1	Pentacosene	28.0	25.1	26.4	27.6	27.5	25.8	28.3	26.0	26.9	27.0
	Pentacosane	28.9	20.7	27.7	25.5	28.2	26.4	24.7	24.2	31.6	30.7
	3 Me C25	25.9	23.4	26.7	26.9	27.2	24.8	25.8	23.1	26.6	25.3
	Heptacosene	29.8	30.8	26.1	31.4	21.2	31.4	37.9	25.1	27.3	34.1
	Heptacosane	33.9	34.7	36.4	36.2	31.5	25.0	32.5	28.8	28.9	25.4
Percentage of M+2	Pentacosene	3.5	3.7	3.0	4.4	3.9	3.9	3.7	4.5	4.1	4.5
	Pentacosane	5.0	4.8	5.0	3.6	5.7	2.9	4.3	3.9	5.9	3.3
	3 Me C25	3.9	3.9	3.1	3.4	4.5	3.9	2.6	3.8	4.5	3.1
	Heptacosene	3.7	5.2	5.2	5.7	4.2	4.7	6.5	4.3	4.1	5.6
	Heptacosane	7.8	7.8	4.6	7.3	7.9	4.9	8.5	5.9	5.5	7.9

0
1-20
20-40
40-60
60-80
80-100
100+

Figure B.6: Data sheet showing the raw control data for the preliminary sodium [2-^{13}C]acetate experiment for *Formica lemani*.

Sodium [2-^{13}C]acetate		*Formica lemani*									
		Sub 1	Sub 2	Sub 3	Sub 4	Sub 5	Sub 6	Sub 7	Sub 8	Sub 9	Sub 10
Abundance of M	Pentacosene	126128	110008	77032	169408	180224	133184	203648	169216	384576	141120
	Pentacosane	21032	31192	24272	23344	24208	17432	19248	31184	31232	17528
	3 Me C25	65856	51984	46624	92008	90808	65960	88976	84488	209280	81648
	Heptacosene	30752	26600	18104	39760	37736	40608	56240	35784	88552	44264
	Heptacosane	6982	7927	7920	5003	4410	3843	4380	9227	7265	3168
Abundance of M+1	Pentacosene	36136	32328	25064	54040	56984	45608	64632	50488	115872	41936
	Pentacosane	7038	8567	7516	6724	6295	5701	7317	9009	8769	6221
	3 Me C25	16856	17232	11517	23408	24568	19912	24424	25920	57224	24096
	Heptacosene	12546	9607	6308	15931	13399	11893	23424	14251	32072	17416
	Heptacosane	2168	3702	2670	1099	2180	1172	1920	4360	2097	1835
Abundance of M+2	Pentacosene	9488	9483	4820	12203	13239	8939	18520	14600	20808	1157
	Pentacosane	958	1096	1662	1670	1149	1347	2118	3663	1947	2242
	3 Me C25	4088	3302	2268	6868	6410	4546	5663	6301	12747	5343
	Heptacosene	4152	4355	2726	5589	4505	3414	7826	3275	9598	6132
	Heptacosane	392	1550	589	430	287	322	451	572	769	613
Percentage of M+1	Pentacosene	28.7	29.4	32.5	31.9	31.6	34.2	31.7	29.8	30.1	29.7
	Pentacosane	33.5	27.5	31.0	28.8	26.0	32.7	38.0	28.9	28.1	35.5
	3 Me C25	25.6	33.1	24.7	25.4	27.1	30.2	27.5	30.7	27.3	29.5
	Heptacosene	40.8	36.1	34.8	40.1	35.5	29.3	41.7	39.8	36.2	39.3
	Heptacosane	31.1	46.7	33.7	22.0	49.4	30.5	43.8	47.3	28.9	57.9
Percentage of M+2	Pentacosene	7.5	8.6	6.3	7.2	7.3	6.7	9.1	8.6	5.4	0.8
	Pentacosane	4.6	3.5	6.8	7.2	4.7	7.7	11.0	11.7	6.2	12.8
	3 Me C25	6.2	6.4	4.9	7.5	7.1	6.9	6.4	7.5	6.1	6.5
	Heptacosene	13.5	16.4	15.1	14.1	11.9	8.4	13.9	9.2	10.8	13.9
	Heptacosane	5.6	19.6	7.4	8.6	6.5	8.4	10.3	6.2	10.6	19.3

0
1-20
20-40
40-60
60-80
80-100
100+

Figure B.7: Data sheet showing the raw substrate data for the preliminary sodium [2-^{13}C]acetate experiment for *Formica lemani*.

Sodium acetate (Control)		*Myrmica sabuleti*									
		Con 1	Con 2	Con 3	Con 4	Con 5	Con 6	Con 7	Con 8	Con 9	Con 10
Abundance of M	Tricosane	134016	176320	98504	188736	56408	70432	17504	39176	38392	82464
	Pentacosene	766656	1346048	548096	671424	441152	300736	156288	302656	301440	525888
	Pentacosane	113648	102072	82296	135104	58312	65424	17504	33152	29384	49600
	5 Me C25	976192	1460736	516032	749184	499456	349504	201984	341760	338432	603008
	Heptacosene	193600	269056	115744	152832	113960	51008	34096	70240	59744	108624
Abundance of M+1	Tricosane	32608	43048	21064	46960	16672	16896	4451	11736	10291	23280
	Pentacosene	190976	339840	151104	165120	124768	69552	37392	77744	77536	137536
	Pentacosane	35848	34424	21840	29208	17008	18912	4454	9882	8889	14586
	5 Me C25	228544	301312	115792	159808	110176	61392	42192	77024	73640	133248
	Heptacosene	49224	80040	36096	34080	35320	15000	12198	21272	16800	32784
Abundance of M+2	Tricosane	2506	6132	3593	5793	1747	1712	1255	1044	2400	3283
	Pentacosene	26032	42096	21816	21016	16284	9124	5332	9436	11487	16208
	Pentacosane	3056	4014	5264	2986	2853	1796	1276	1596	1256	1848
	5 Me C25	25568	29720	14531	21808	12541	8086	3312	8154	10971	15990
	Heptacosene	9280	11069	6330	6308	5663	1598	455	3576	3099	8677
Percentage of M+1	Tricosane	24.3	24.4	21.4	24.9	29.6	24.0	25.4	30.0	26.8	28.2
	Pentacosene	24.9	25.2	27.6	24.6	28.3	23.1	23.9	25.7	25.7	26.2
	Pentacosane	31.5	33.7	26.5	21.6	29.2	28.9	25.4	29.8	30.3	29.4
	5 Me C25	23.4	20.6	22.4	21.3	22.1	17.6	20.9	22.5	21.8	22.1
	Heptacosene	25.4	29.7	31.2	22.3	31.0	29.4	35.8	30.3	28.1	30.2
Percentage of M+2	Tricosane	1.9	3.5	3.6	3.1	3.1	2.4	7.2	2.7	6.3	4.0
	Pentacosene	3.4	3.1	4.0	3.1	3.7	3.0	3.4	3.1	3.8	3.1
	Pentacosane	2.7	3.9	6.4	2.2	4.9	2.7	7.3	4.8	4.3	3.7
	5 Me C25	2.6	2.0	2.8	2.9	2.5	2.3	1.6	2.4	3.2	2.7
	Heptacosene	4.8	4.1	5.5	4.1	5.0	3.1	1.3	5.1	5.2	8.0

0
1-20
20-40
40-60
60-80
80-100
100+

Figure B.8: Data sheet showing the raw control data for the preliminary sodium [1-^{13}C]acetate experiment for *Myrmica sabuleti*.

Sodium [1-^{13}C]acetate		*Myrmica sabuleti*									
		Sub 1	Sub 2	Sub 3	Sub 4	Sub 5	Sub 6	Sub 7	Sub 8	Sub 9	Sub 10
Abundance of M	Tricosane	74408	60688	66432	86400	67616	88944	96256	96256	76944	74648
	Pentacosene	335232	243136	322944	535232	235456	413760	469376	461632	476160	445888
	Pentacosane	57944	48944	42160	75704	58992	66952	33720	67360	50360	50896
	5 Me C25	499136	393152	470528	716608	402240	608000	648512	695040	651328	663616
	Heptacosene	65040	35960	65088	101904	50720	95584	87720	95824	95696	90336
Abundance of M+1	Tricosane	29104	43232	37272	46808	43472	61248	59328	59328	55768	47840
	Pentacosene	160384	133824	185536	285440	153088	235776	245824	279488	306304	305152
	Pentacosane	27832	29040	25624	40752	35448	35184	22216	51680	34872	34848
	5 Me C25	183808	183232	223808	314560	258176	310912	292096	352640	354560	337536
	Heptacosene	39224	28328	39256	58848	36680	51928	48912	61840	78064	60512
Abundance of M+2	Tricosane	16012	15690	17184	19280	24296	24016	19408	19408	27888	26384
	Pentacosene	68784	59752	83048	131456	93232	107072	112280	136832	157952	15078
	Pentacosane	7613	13751	13974	16504	22464	19904	5482	22680	20840	17424
	5 Me C25	75496	81608	87304	105104	123728	121848	104256	139712	153088	144192
	Heptacosene	12916	12439	18472	29608	17272	33792	29760	35880	37160	37808
Percentage of M+1	Tricosane	39.1	71.2	56.1	54.2	64.3	68.9	61.6	61.6	72.5	64.1
	Pentacosene	47.8	55.0	57.5	53.3	65.0	57.0	52.4	60.5	64.3	68.4
	Pentacosane	48.0	59.3	60.8	53.8	60.1	52.6	65.9	76.7	69.2	68.5
	5 Me C25	36.8	46.6	47.6	43.9	64.2	51.1	45.0	50.7	54.4	50.9
	Heptacosene	60.3	78.8	60.3	57.7	72.3	54.3	55.8	64.5	81.6	67.0
Percentage of M+2	Tricosane	21.5	25.9	25.9	22.3	35.9	27.0	20.2	20.2	36.2	35.3
	Pentacosene	20.5	24.6	25.7	24.6	39.6	25.9	23.9	29.6	33.2	3.4
	Pentacosane	13.1	28.1	33.1	21.8	38.1	29.7	16.3	33.7	41.4	34.2
	5 Me C25	15.1	20.8	18.6	14.7	30.8	20.0	16.1	20.1	23.5	21.7
	Heptacosene	19.9	34.6	28.4	29.1	34.1	35.4	33.9	37.4	38.8	41.9

0
1-20
20-40
40-60
60-80
80-100
100+

Figure B.9: Data sheet showing the raw substrate data for the preliminary sodium [1-^{13}C]acetate experiment for *Myrmica sabuleti*.

Sodium [2-^{13}C]acetate		*Myrmica sabuleti*									
		Con 1	Con 2	Con 3	Con 4	Con 5	Con 6	Con 7	Con 8	Con 9	Con 10
Abundance of M	Tricosane	11928	36800	29496	14938	10311	31112	15017	24000	15019	32944
	Pentacosene	60184	98792	22728	56920	30384	97808	43384	32952	67472	69944
	Pentacosane	17064	25936	37272	20768	12335	24592	26336	24544	23264	34472
	5 Me C25	65904	118792	47264	66456	4228	116024	65296	86064	84768	112488
	Heptacosene	16361	23216	6270	13383	9541	24256	16337	17544	27712	17864
Abundance of M+1	Tricosane	3713	8366	6031	3334	3524	7222	3820	7653	4937	8386
	Pentacosene	13372	27048	5757	15697	7979	29656	12522	10304	18248	18096
	Pentacosane	4177	6253	8091	4714	4034	4895	6840	6780	7561	9370
	5 Me C25	14611	25000	9358	12726	655	23512	12692	18640	17856	24768
	Heptacosene	4699	7380	1710	3942	2723	8758	4122	4463	9315	5883
Abundance of M+2	Tricosane	1501	592	624	618	187	566	456	485	876	1080
	Pentacosene	1534	3010	973	1912	856	4293	1789	821	2878	2402
	Pentacosane	905	438	1581	763	744	972	1200	758	1029	1170
	5 Me C25	1858	3151	1646	1702	166	2823	1919	2096	2701	2480
	Heptacosene	893	896	502	551	541	2483	863	1321	1096	743
Percentage of M+1	Tricosane	31.1	22.7	20.4	22.3	34.2	23.2	25.4	31.9	32.9	25.5
	Pentacosene	22.2	27.4	25.3	27.6	26.3	30.3	28.9	31.3	27.0	25.9
	Pentacosane	24.5	24.1	21.7	22.7	32.7	19.9	26.0	27.6	32.5	27.2
	5 Me C25	22.2	21.0	19.8	19.1	15.5	20.3	19.4	21.7	21.1	22.0
	Heptacosene	28.7	31.8	27.3	29.5	28.5	36.1	25.2	25.4	33.6	32.9
Percentage of M+2	Tricosane	12.6	1.6	2.1	4.1	1.8	1.8	3.0	2.0	5.8	3.3
	Pentacosene	2.5	3.0	4.3	3.4	2.8	4.4	4.1	2.5	4.3	3.4
	Pentacosane	5.3	1.7	4.2	3.7	6.0	4.0	4.6	3.1	4.4	3.4
	5 Me C25	2.8	2.7	3.5	2.6	3.9	2.4	2.9	2.4	3.2	2.2
	Heptacosene	5.5	3.9	8.0	4.1	5.7	10.2	5.3	7.5	4.0	4.2

0
1-20
20-40
40-60
60-80
80-100
100+

Figure B.10: Data sheet showing the raw control data for the preliminary sodium [2-^{13}C]acetate experiment for *Myrmica sabuleti*.

Sodium [2-^{13}C]acetate		*Myrmica sabuleti*									
		Sub 1	Sub 2	Sub 3	Sub 4	Sub 5	Sub 6	Sub 7	Sub 8	Sub 9	Sub 10
Abundance of M	Tricosane	4907	2308	3040	6795	2853	5611	8302	9372	2751	1702
	Pentacosene	8901	5470	4706	43520	3968	10979	18352	8492	4683	13577
	Pentacosane	6528	7013	2914	7079	5620	7996	8453	20920	4376	2194
	5 Me C25	26960	18088	13558	78808	9717	32952	42584	19216	14305	25048
	Heptacosene	3012	1058	1376	13647	662	4073	4776	2888	2016	2809
Abundance of M+1	Tricosane	4191	1220	1387	5968	1708	5593	4961	5019	1462	1302
	Pentacosene	12226	3436	5552	31288	2495	13106	14676	7964	5777	12971
	Pentacosane	2162	2619	1352	5503	1541	5416	3921	7818	1490	542
	5 Me C25	22752	13740	10027	49152	8528	27912	31824	16840	13934	17936
	Heptacosene	2510	1291	1666	10278	1136	3597	3865	2046	1955	2821
Abundance of M+2	Tricosane	3386	789	1832	4057	809	5091	4129	4301	1315	1765
	Pentacosene	11386	3517	4159	23008	1648	11544	13902	6007	3930	10217
	Pentacosane	1808	1206	1552	1936	1277	3561	2765	2433	1097	736
	5 Me C25	16632	9342	8807	30408	5833	20288	21320	11292	9936	15392
	Heptacosene	1403	1119	1726	7289	788	3193	3493	1764	1700	2703
Percentage of M+1	Tricosane	85.4	52.9	45.6	87.8	59.9	99.7	59.8	53.6	53.1	76.5
	Pentacosene	137.4	62.8	118.0	71.9	62.9	119.4	80.0	93.8	123.4	95.5
	Pentacosane	33.1	37.3	46.4	77.7	27.4	67.7	46.4	37.4	34.0	24.7
	5 Me C25	84.4	76.0	74.0	62.4	87.8	84.7	74.7	87.6	97.4	71.6
	Heptacosene	83.3	122.0	121.1	75.3	171.6	88.3	80.9	70.8	97.0	100.4
Percentage of M+2	Tricosane	69.0	34.2	60.3	59.7	28.4	90.7	49.7	45.9	47.8	103.7
	Pentacosene	127.9	64.3	88.4	52.9	41.5	105.1	75.8	70.7	83.9	75.3
	Pentacosane	27.7	17.2	53.3	27.3	22.7	44.5	32.7	11.6	25.1	33.5
	5 Me C25	61.7	51.6	65.0	38.6	60.0	61.6	50.1	58.8	69.5	61.5
	Heptacosene	46.6	105.8	125.4	53.4	119.0	78.4	73.1	61.1	84.3	96.2

0
1-20
20-40
40-60
60-80
80-100
100+

Figure B.11: Data sheet showing the raw substrate data for the preliminary sodium [2-^{13}C]acetate experiment for *Myrmica sabuleti*.

Propionic Acid (Control)		*Formica lugubris*													
		Con 1	Con 2	Con 3	Con 4	Con 5	Con 6	Con 7	Con 8	Con 9	Con 10	Con 11	Con 12	Con 13	Con 14
Abundance of M	Pentacosene	13720	3491	8782	7022	9571	1075	5422	6062	8521	11182	3681	2194	3098	10833
	Pentacosane	29848	3780	11101	6989	12806	3659	1780	2775	11512	4408	4267	10847	4127	10620
	5 Me C25	19144	3741	13675	24704	20752	3470	10856	20160	14606	19368	7686	1842	3784	10780
	Heptacosene	35576	8008	24272	9922	29736	6662	20008	38064	27560	25896	17168	7760	8811	31360
	Heptacosane	39104	2689	9678	10893	24488	4078	2147	2349	12453	3428	4130	13830	5646	20944
	Nonacosene	48416	10401	36080	36960	37336	8119	26264	42024	37016	34368	15618	10587	14237	35352
	Nonacosane	37248	1607	7836	12269	27168	1853	1981	864	9966	1811	2845	11258	4710	29856
	11 13 15 C29	28832	7485	15974	12712	20432	6010	16984	23208	14566	15668	10660	2922	5796	14933
Abundance of M+1	Pentacosene	3021	780	2786	2131	3036	297	1865	1895	3285	2088	1574	1330	682	3007
	Pentacosane	8665	1290	3513	1872	4454	1035	670	1340	2574	1094	1261	3269	2027	4332
	5 Me C25	4770	1095	3449	6419	4036	547	3386	4553	2555	2391	1710	459	1226	2322
	Heptacosene	9724	2279	6900	3953	9331	1832	5702	12940	8261	7690	3867	1790	2295	8469
	Heptacosane	12125	843	2850	4140	6984	1948	219	1223	4487	1700	991	3729	1903	7026
	Nonacosene	16092	3377	9975	11451	13554	2243	7744	8833	12164	11066	7055	3785	3986	13044
	Nonacosane	11621	682	1633	3076	7943	890	194	146	3703	620	1390	4395	1518	9039
	11 13 15 C29	22144	4171	14333	11842	16222	4285	9572	13826	9237	12604	5820	1214	2791	9259
Abundance of M+2	Pentacosene	210	299	297	450	862	44	401	626	288	447	187	245	0	441
	Pentacosane	1018	716	342	598	701	597	0	0	378	0	271	319	222	309
	5 Me C25	544	195	370	845	476	0	230	0	244	265	284	0	0	205
	Heptacosene	1897	458	696	251	1197	503	1005	1480	957	1627	426	216	619	1315
	Heptacosane	1958	183	300	264	1377	0	0	228	1554	0	0	767	463	1006
	Nonacosene	2991	422	1546	1582	1820	649	1399	2418	2288	2244	1161	563	681	1760
	Nonacosane	2046	483	604	741	1645	416	0	122	315	0	0	1349	0	1088
	11 13 15 C29	1625	980	1277	1241	1015	283	1369	1539	2256	1447	1098	0	682	926
Percentage of M+1	Pentacosene	22.0	22.3	31.7	30.3	31.7	27.6	34.4	31.3	38.6	18.7	42.8	60.6	22.0	27.8
	Pentacosane	29.0	34.1	31.6	26.8	34.8	28.3	37.6	48.3	22.4	24.8	29.6	30.1	49.1	40.8
	5 Me C25	24.9	29.3	25.2	26.0	19.4	15.8	31.2	22.6	17.5	12.3	22.2	24.9	32.4	21.5
	Heptacosene	27.3	28.5	28.4	39.8	31.4	27.5	28.5	34.0	30.0	29.7	22.5	23.1	26.0	27.0
	Heptacosane	31.0	31.3	29.4	38.0	28.5	47.8	10.2	52.1	36.0	49.6	24.0	27.0	33.7	33.5
	Nonacosene	33.2	32.5	27.6	31.0	36.3	27.6	29.5	21.0	32.9	32.2	45.2	35.8	28.0	36.9
	Nonacosane	31.2	42.4	20.8	25.1	29.2	48.0	9.8	16.9	37.2	34.2	48.9	39.0	32.2	30.3
	11 13 15 C29	76.8	55.7	89.7	93.2	79.4	71.3	56.4	59.6	63.4	80.4	54.6	41.5	48.2	62.0
Percentage of M+2	Pentacosene	1.5	8.6	3.4	6.4	9.0	4.1	7.4	10.3	3.4	4.0	5.1	11.2	0.0	4.1
	Pentacosane	3.4	18.9	3.1	8.6	5.5	16.3	0.0	0.0	3.3	0.0	6.4	2.9	5.4	2.9
	5 Me C25	2.8	5.2	2.7	3.4	2.3	0.0	2.1	0.0	1.7	1.4	3.7	0.0	0.0	1.9
	Heptacosene	5.3	5.7	2.9	2.5	4.0	7.6	5.0	3.9	3.5	6.3	2.5	2.8	7.0	4.2
	Heptacosane	5.0	6.8	3.1	2.4	5.6	0.0	0.0	9.7	12.5	0.0	0.0	5.5	8.2	4.8
	Nonacosene	6.2	4.1	4.3	4.3	4.9	8.0	5.3	5.8	6.2	6.5	7.4	5.3	4.8	5.0
	Nonacosane	5.5	30.1	7.7	6.0	6.1	22.5	0.0	14.1	3.2	0.0	0.0	12.0	0.0	3.6
	11 13 15 C29	5.6	13.1	8.0	9.8	5.0	4.7	8.1	6.6	15.5	9.2	10.3	0.0	11.8	6.2

0
1-20
20-40
40-60
60-80
80-100
100+

Figure B.12: Data sheet showing the raw control data for the preliminary propionate experiment for *Formica lugubris*.

Propionic-2,2-d_2 acid-d		*Formica lugubris*																			
		P2: 1	P2: 2	P2: 3	P2: 4	P2: 5	P2: 6	P2: 7	P2: 8	P2: 9	P2: 10	P2: 11	P2: 12	P2: 13	P2: 14	P2: 15	P2: 16	P2: 17	P2: 18	P2: 19	P2: 20
Abundance of M	Tricosene	1957	1625	5840	2464	1341	17984	0	4778	4996	1971	3620	1744	0	1924	4293	5854		12970	5826	1323
	Tricosane	2001	2464	6470	11089	1956	36128	20296	19072	15438	21136	5051	13661	6875	5410	5294	20088		28560	14866	8855
	3 Me C23	7091	6366	19088	5302	6606	16672	1802	10597	10769	6350	15280	0	0	3406	15796	6637		20680	16680	392
	Pentacosene	6698	5694	15214	5615	4869	32904	3209	18592	15438	5714	13032	14210	999	3872	16664	9169		40400	25296	3262
	Pentacosane	1878	3147	12752	16704	2509	33528	20552	23696	23024	22504	6058	14687	10123	7977	24136	24136		24328	18224	7791
	3 Me C25	8055	6318	34360	8704	4129	47584	2173	34152	33432	10429	20088	21144	1344	6550	23720	19656		51208	47592	3943
	Heptacosene	2312	1178	7589	20816	1258	31440	26632	20952	22272	14123	3838	14719	8412	10529	4866	13288		24328	18520	9077
	Heptacosane	9176	4813	26648	10003	9971	19944	3684	14144	16073	8027	30800	17592	0	3373	14371	11779		21240	16824	2969
Abundance of M+1	Tricosene	277	689	1013	570	757	4529	0	1286	1860	559	2803	339	0	466	1926	1558		6064	1653	497
	Tricosane	558	1323	1968	3114	833	9170	5543	4765	3475	4821	1490	3726	2807	1395	2102	4619		6210	2490	2430
	3 Me C23	1661	2345	5290	1294	1879	2515	307	3079	4957	1677	3721	0	0	911	3328	2226		4795	4196	0
	Pentacosene	2404	1666	6661	1241	878	8558	452	5570	4073	1292	4550	3160	255	1426	5155	3509		13015	7090	1507
	Pentacosane	895	1017	2569	4149	420	11735	5192	6535	5782	8199	1478	5834	3021	3701	7808	7808		6014	6256	3105
	3 Me C25	3432	1774	8815	2095	2118	15391	861	12152	11842	4338	7012	5210	656	1465	7344	4952		13249	16776	1172
	Heptacosene	673	769	3455	5291	299	9504	9353	6514	8924	4621	2264	4019	2191	4615	1180	6482		6014	7222	3271
	Heptacosane	6295	3884	16472	6382	5736	12847	2238	10501	11943	4137	21520	11370	0	2857	9372	6060		14666	12938	2569
Abundance of M+2	Tricosene	0	0	541	0	0	235	0	378	419	196	0	0	0	0	0	387		616	452	0
	Tricosane	311	406	276	0	0	1611	745	1226	627	1244	0	655	656	199	725	813		1678	1099	0
	3 Me C23	0	239	714	0	0	874	0	526	184	410	515	0	0	0	709	0		186	320	0
	Pentacosene	0	315	1079	487	0	2198	0	1204	774	616	970	532	0	713	810	783		1342	1308	367
	Pentacosane	0	673	501	354	0	1474	1117	1263	1119	1052	452	533	346	1244	490	1756		1291	540	0
	3 Me C25	0	0	1420	632	315	2975	150	1751	1701	282	1004	1163	0	0	1728	2186		1952	2739	0
	Heptacosene	0	0	482	920	0	2562	1792	1113	665	407	242	1102	372	536	0	744		1291	1853	329
	Heptacosane	659	604	1721	670	1346	1845	0	1061	2423	1037	3126	2163	0	1344	1888	1122		2695	1532	0
Percentage of M+1	Tricosene	14.2	42.4	17.3	23.1	56.5	25.2		26.9	37.2	28.4	77.4	19.4		24.2	44.9	26.6		46.8	28.4	37.6
	Tricosane	27.9	53.7	30.4	28.1	42.6	25.4	27.3	25.0	22.5	22.8	29.5	27.3	40.8	25.8	39.7	23.0		21.7	16.7	27.4
	3 Me C23	23.4	36.8	27.7	24.4	28.4	15.1	17.0	29.1	46.0	26.4	24.4			26.7	21.1	33.5		23.2	25.2	0.0
	Pentacosene	35.9	29.3	43.8	22.1	18.0	26.0	14.1	30.0	26.4	22.6	34.9	22.2	25.5	36.8	30.9	38.3		32.2	28.0	46.2
	Pentacosane	47.7	32.3	20.1	24.8	16.7	35.0	25.3	27.6	25.1	36.4	24.4	39.7	29.8	46.4	32.4	32.4		24.7	34.3	39.9
	3 Me C25	42.6	28.1	25.7	24.1	51.3	32.3	39.6	35.6	35.4	41.6	34.9	24.6	48.8	22.4	31.0	25.2		25.9	35.2	29.7
	Heptacosene	29.1	65.3	45.5	25.4	23.8	30.2	35.1	31.1	40.1	32.7	59.0	27.3	26.0	43.8	24.2	48.8		24.7	39.0	36.0
	Heptacosane	68.6	80.7	61.8	63.8	57.5	64.4	60.7	74.2	74.3	51.5	69.9	64.6		84.7	65.2	51.4		69.0	76.9	86.5
Percentage of M+2	Tricosene	0.0	0.0	9.3	0.0	0.0	1.3		7.9	8.4	9.9	0.0	0.0		0.0	0.0	6.6		4.7	7.8	0.0
	Tricosane	15.5	16.5	4.3	0.0	0.0	4.5	3.7	6.4	4.1	5.9	0.0	4.8	9.5	3.7	13.7	4.0		5.9	7.4	0.0
	3 Me C23	0.0	3.8	3.7	0.0	0.0	5.2	0.0	5.0	1.7	6.5	3.4			0.0	4.5	0.0		0.9	1.9	0.0
	Pentacosene	0.0	5.5	7.1	8.7	0.0	6.7	0.0	6.5	5.0	10.8	7.4	3.7	0.0	18.4	4.9	8.5		3.3	5.2	11.3
	Pentacosane	0.0	21.4	3.9	2.1	0.0	4.4	5.4	5.3	4.9	4.7	7.5	3.6	3.4	15.6	2.0	7.3		5.3	3.0	0.0
	3 Me C25	0.0	0.0	4.1	7.3	7.6	6.3	6.9	5.1	5.1	2.7	5.0	5.5	0.0	0.0	7.3	11.1		3.8	5.8	0.0
	Heptacosene	0.0	0.0	6.4	4.4	0.0	8.1	6.7	5.3	3.0	2.9	6.3	7.5	4.4	5.1	0.0	5.6		5.3	10.0	3.6
	Heptacosane	7.2	12.5	6.5	6.7	13.5	9.3	0.0	7.5	15.1	12.9	10.1	12.3		39.8	13.1	9.5		12.7	9.1	0.0

0
1-20
20-40
40-60
60-80
80-100
100+

Figure B.13: Data sheet showing the raw propionic-2,2-d_2 acid-d data for the preliminary propionate experiment for *Formica lugubris*. Note that there was insufficient data for analysis of sodium [1-^{13}C]propionate so it is not shown here.

Propionic Acid (Control)		*Formica lemani*																			
		Con 1	Con 2	Con 3	Con 4	Con 5	Con 6	Con 7	Con 8	Con 9	Con 10	Con 11	Con 12	Con 13	Con 14	Con 15	Con 16	Con 17	Con 18	Con 19	Con 20
Abundance of M	Tricosene	26216	16592	16640	9529	3622	11917	21920	18984	4579	12447	11219	16824	9636	27704	12468	10149	34568	17112	18080	7774
	Tricosane	7621	8401	11333	4758	1931	4855	12355	4321	3659	7698	4296	13638	6989	8649	7503	4679	13491	5875	6439	3088
	3 Me C23	62232	29144	54272	32736	15810	21368	74240	48976	10487	32040	24488	42488	16576	71936	41240	24360	71256	44384	52160	16237
	Pentacosene	512896	339072	504384	287232	168000	247744	595968	399616	150464	358976	276096	383168	215360	522240	365888	259456	621952	394240	445376	189440
	Pentacosane	18856	35216	13326	8621	4334	10712	17296	9493	8157	18072	12115	29560	19176	20888	10519	17448	20168	9630	10048	7512
	3 Me C25	190592	122472	196480	111784	56664	96496	236416	131264	54376	128056	101720	153408	70072	212928	140352	108808	245952	151744	166400	66776
	Heptacosene	206912	132608	214080	122192	69552	100760	242688	152064	66000	131040	116912	188032	88984	206848	155328	113144	226816	165952	170944	75352
	Heptacosane	3249	9216	3122	2192	1180	3201	4743	1084	2950	5125	2416	7717	5694	6237	2592	3282	2860	3271	1931	2889
Abundance of M+1	Tricosene	5531	5351	4738	2493	1266	3597	5098	3437	662	3622	2212	5377	2751	6256	4433	1399	6607	3292	3889	1872
	Tricosane	1535	1249	2108	1566	610	1803	3614	1476	541	1382	1050	2396	1813	2818	2536	1618	3256	881	1354	868
	3 Me C23	14750	6842	14172	5903	3597	2832	19496	10606	2703	9354	6397	9924	3985	17936	9457	7578	16352	7988	10474	2805
	Pentacosene	136320	88584	131968	70144	42536	64512	155392	98064	42576	86224	71536	106312	56648	141568	97368	66304	157632	105256	113912	54704
	Pentacosane	4882	9932	3512	2773	1686	3057	6542	2013	1739	4041	4032	8942	4599	5542	2438	7127	5799	3126	3572	2659
	3 Me C25	49464	30016	46944	24808	15650	22560	53280	30632	10140	33096	25616	38568	19160	53760	33440	22824	63368	40360	39664	18024
	Heptacosene	61400	39784	56176	36712	15798	29896	68128	44072	21600	33424	30656	53720	24872	63784	41552	33792	66560	45320	45048	21496
	Heptacosane	1265	2482	897	242	151	791	958	230	794	1602	409	2410	1755	1544	601	905	481	1438	670	954
Abundance of M+2	Tricosene	616	311	839	204	342	529	1009	191	0	930	282	667	151	898	654	208	1081	934	537	717
	Tricosane	233	264	304	0	179	0	169	237	0	0	0	0	0	244	0	254	353	243	0	0
	3 Me C23	2225	525	2269	938	510	252	1070	1133	186	1128	481	1658	1100	2397	1343	495	1834	923	1061	320
	Pentacosene	14453	10850	16744	9931	6453	7450	19944	14962	5199	9385	9408	12336	9290	15238	15504	8414	1312	12915	13745	6278
	Pentacosane	927	865	965	624	334	405	674	0	192	834	444	1204	1334	607	378	797	700	563	700	459
	3 Me C25	7202	5223	6746	3318	0	4128	7824	3217	1106	3476	2235	4908	1738	6134	3695	4065	8414	3905	5183	2022
	Heptacosene	6146	4601	5519	4462	3364	4308	9013	5967	2421	5626	3525	5358	3043	8295	6783	4859	10514	8108	6151	3760
	Heptacosane	306	392	202	0	0	0	290	0	0	311	0	246	464	0	0	0	209	184	151	0
Percentage of M+1	Tricosene	21.1	32.3	28.5	26.2	35.0	30.2	23.3	18.1	14.5	29.1	19.7	32.0	28.5	22.6	35.6	13.8	19.1	19.2	21.5	24.1
	Tricosane	20.1	14.9	18.6	32.9	31.6	37.1	29.3	34.2	14.8	18.0	24.4	17.6	25.9	32.6	33.8	34.6	24.1	15.0	21.0	28.1
	3 Me C23	23.7	23.5	26.1	18.0	22.8	13.3	26.3	21.7	25.8	29.2	26.1	23.4	24.0	24.9	22.9	31.1	22.9	18.0	20.1	17.3
	Pentacosene	26.6	26.1	26.2	24.4	25.3	26.0	26.1	24.5	28.3	24.0	25.9	27.7	26.3	27.1	26.6	25.6	25.3	26.7	25.6	28.9
	Pentacosane	25.9	28.2	26.4	32.2	38.9	28.5	37.8	21.2	21.3	22.4	33.3	30.3	24.0	26.5	23.2	40.8	28.8	32.5	35.5	35.4
	3 Me C25	26.0	24.5	23.9	22.2	27.6	23.4	22.5	23.3	18.6	25.8	25.2	25.1	27.3	25.2	23.8	21.0	25.8	26.6	23.8	27.0
	Heptacosene	29.7	30.0	26.2	30.0	22.7	29.7	28.1	29.0	32.7	25.5	26.2	28.6	28.0	30.8	26.8	29.9	29.3	27.3	26.4	28.5
	Heptacosane	38.9	26.9	28.7	11.0	12.8	24.7	20.2	21.2	26.9	31.3	16.9	31.2	30.8	24.8	23.2	27.6	16.8	44.0	34.7	33.0
Percentage of M+2	Tricosene	2.3	1.9	5.0	2.1	9.4	4.4	4.6	1.0	0.0	7.5	2.5	4.0	1.6	3.2	5.2	2.0	3.1	5.5	3.0	9.2
	Tricosane	3.1	3.1	2.7	0.0	9.3	0.0	1.4	5.5	0.0	0.0	0.0	0.0	0.0	2.8	0.0	5.4	2.6	4.1	0.0	0.0
	3 Me C23	3.6	1.8	4.2	2.9	3.2	1.2	1.4	2.3	1.8	3.5	2.0	3.9	6.6	3.3	3.3	2.0	2.6	2.1	2.0	2.0
	Pentacosene	2.8	3.2	3.3	3.5	3.8	3.0	3.3	3.7	3.5	2.6	3.4	3.2	4.3	2.9	4.2	3.2	0.2	3.3	3.1	3.3
	Pentacosane	4.9	2.5	7.2	7.2	7.7	3.8	3.9	0.0	2.4	4.6	3.7	4.1	7.0	2.9	3.6	4.6	3.5	5.8	7.0	6.1
	3 Me C25	3.8	4.3	3.4	3.0	0.0	4.3	3.3	2.5	2.0	2.7	2.2	3.2	2.5	2.9	2.6	3.7	3.4	2.6	3.1	3.0
	Heptacosene	3.0	3.5	2.6	3.7	4.8	4.3	3.7	3.9	3.7	4.3	3.0	2.8	3.4	4.0	4.4	4.3	4.6	4.9	3.6	5.0
	Heptacosane	9.4	4.3	6.5	0.0	0.0	0.0	6.1	0.0	0.0	6.1	0.0	3.2	8.1	0.0	0.0	0.0	7.3	5.6	7.8	0.0

0
1-20
20-40
40-60
60-80
80-100
100+

Figure B.14: Data sheet showing the raw control data for the preliminary propionate experiment for *Formica lemani*.

Sodium [1-^{13}C]propionate		*Formica lemani*																		
		P1: 1	P1: 2	P1: 3	P1: 4	P1: 5	P1: 6	P1: 7	P1: 8	P1: 9	P1: 10	P1: 11	P1: 12	P1: 13	P1: 14	P1: 15	P1: 16	P1: 17	P1: 18	P1: 19
Abundance of M	Tricosene	5611	20424	9026	7039	16448	19640	9857	19008	9449	18600	10092	18392	20552	25384	20808	6502	32648	8583	6607
	Tricosane	2834	8508	10604	4898	8256	9506	13770	8250	3701	4889	4160	8715	9270	10564	7851	3466	11358	14920	8574
	3 Me C23	13889	38544	14469	17888	31720	28624	8892	35304	12108	31128	14405	39096	43080	34344	41504	12577	49640	16231	8349
	Pentacosene	190400	441600	228096	217664	376064	354880	199488	366592	182411	348288	194688	416320	431040	384576	390080	216896	612672	196864	162112
	Pentacosane	5355	15172	29592	11553	18680	20680	41944	13158	7566	8699	21160	18184	17176	20032	21784	6661	17072	60768	35160
	3 Me C25	50016	136576	63072	75464	125304	115792	54312	124376	47544	107048	50336	139264	142016	138048	124488	67776	190592	66800	39776
	Heptacosene	73968	165056	92904	89416	155648	162816	76896	156480	76776	145472	83888	125136	162688	157696	150528	94224	190592	85648	66104
	Heptacosane	1138	3218	9504	3019	2840	5725	11401	2711	2091	2362	6067	3395	4413	4195	4777	1451	3533	23936	13739
Abundance of M+1	Tricosene	1957	6003	3064	1860	5147	4401	2131	4646	3232	4952	1914	6524	5871	6965	5757	1849	7913	2544	2004
	Tricosane	912	2296	1432	1746	1663	1967	2278	1636	1132	1490	1385	2326	2198	2182	2739	809	3197	3413	1742
	3 Me C23	8290	17376	6683	7896	15242	14412	3944	16960	7281	13549	5952	18608	20768	16448	16840	8663	24448	7160	3892
	Pentacosene	49968	120944	58584	59632	96632	101192	50656	93280	49184	84472	53456	109072	106168	103960	103232	55312	148352	54688	40128
	Pentacosane	1128	4627	7198	3612	4248	4576	9722	3563	2591	1747	4867	4595	4492	5987	6141	1485	4998	16408	10519
	3 Me C25	24976	67352	33024	32080	61496	48984	26840	63024	21672	49008	20328	65296	61368	57912	53920	30512	81184	31712	22160
	Heptacosene	23384	53512	26712	26088	40632	42616	24336	46392	21752	38104	23280	31048	51928	49512	39584	24520	81184	27064	16616
	Heptacosane	411	699	3879	656	1154	2168	5049	1244	609	363	2231	661	944	1247	1339	619	1269	6267	4024
Abundance of M+2	Tricosene	0	749	388	225	0	429	460	933	574	954	433	502	1066	585	1015	743	806	450	242
	Tricosane	0	262	23	342	0	390	257	784	0	389	0	0	550	348	488	413	952	590	314
	3 Me C23	1262	4461	1115	1833	3238	2670	1102	2818	1449	1984	1301	3749	3563	2149	3287	365	3971	1519	708
	Pentacosene	8003	14485	7297	7342	15230	14455	7994	13412	7233	12958	7790	14111	13503	12587	9725	7323	18704	7403	3615
	Pentacosane	457	443	2530	395	827	784	624	877	332	643	335	414	718	1094	443	263	1392	2231	0
	3 Me C25	4549	13530	5535	5678	10076	8507	4649	10297	4785	8255	3960	15445	12106	9616	9925	7445	13612	6945	3500
	Heptacosene	3341	6655	4339	5103	5552	4697	3725	5612	3169	4960	2403	4588	7338	4745	7865	3634	13612	3696	2817
	Heptacosane	0	245	292	0	0	0	0	0	0	0	0	379	0	0	0	0	163	1732	477
Percentage of M+1	Tricosene	34.9	29.4	33.9	26.4	31.3	22.4	21.6	24.4	34.2	26.6	19.0	35.5	28.6	27.4	27.7	28.4	24.2	29.6	30.3
	Tricosane	32.2	27.0	13.5	35.6	20.1	20.7	16.5	19.8	30.6	30.5	33.3	26.7	23.7	20.7	34.9	23.3	28.1	22.9	20.3
	3 Me C23	59.7	45.1	46.2	44.1	48.1	50.3	44.4	48.0	60.1	43.5	41.3	47.6	48.2	47.9	40.6	68.9	49.3	44.1	46.6
	Pentacosene	26.2	27.4	25.7	27.4	25.7	28.5	25.4	25.4	27.0	24.3	27.5	26.2	24.6	27.0	26.5	25.5	24.2	27.8	24.8
	Pentacosane	21.1	30.5	24.3	31.3	22.7	22.1	23.2	27.1	34.2	20.1	23.0	25.3	26.2	29.9	28.2	22.3	29.3	27.0	29.9
	3 Me C25	49.9	49.3	52.4	42.5	49.1	42.3	49.4	50.7	45.6	45.8	40.4	46.9	43.2	42.0	43.3	45.0	42.6	47.5	55.7
	Heptacosene	31.6	32.4	28.8	29.2	26.1	26.2	31.6	29.6	28.3	26.2	27.8	24.8	31.9	31.4	26.3	26.0	42.6	31.6	25.1
	Heptacosane	36.1	21.7	40.8	21.7	40.6	37.9	44.3	45.9	29.1	15.4	36.8	19.5	21.4	29.7	28.0	42.7	35.9	26.2	29.3
Percentage of M+2	Tricosene	0.0	3.7	4.3	3.2	0.0	2.2	4.7	4.9	6.1	5.1	4.3	2.7	5.2	2.3	4.9	11.4	2.5	5.2	3.7
	Tricosane	0.0	3.1	0.2	7.0	0.0	4.1	1.9	9.5	0.0	8.0	0.0	0.0	5.9	3.3	6.2	11.9	8.4	4.0	3.7
	3 Me C23	9.1	11.6	7.7	10.2	10.2	9.3	12.4	8.0	12.0	6.4	9.0	9.6	8.3	6.3	7.9	2.9	8.0	9.4	8.5
	Pentacosene	4.2	3.3	3.2	3.4	4.0	4.1	4.0	3.7	4.0	3.7	4.0	3.4	3.1	3.3	2.5	3.4	3.1	3.8	2.2
	Pentacosane	8.5	2.9	8.5	3.4	4.4	3.8	1.5	6.7	4.4	7.4	1.6	2.3	4.2	5.5	2.0	3.9	8.2	3.7	0.0
	3 Me C25	9.1	9.9	8.8	7.5	8.0	7.3	8.6	8.3	10.1	7.7	7.9	11.1	8.5	7.0	8.0	11.0	7.1	10.4	8.8
	Heptacosene	4.5	4.0	4.7	5.7	3.6	2.9	4.8	3.6	4.1	3.4	2.9	3.7	4.5	3.0	5.2	3.9	7.1	4.3	4.3
	Heptacosane	0.0	7.6	3.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	11.2	0.0	0.0	0.0	0.0	4.6	7.2	3.5

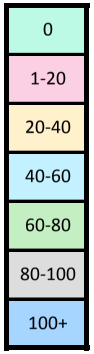


Figure B.15: Data sheet showing the raw sodium [1-^{13}C]propionate data for the preliminary propionate experiment for *Formica lemani*.

Propionic-2,2-d_2 acid-d		*Formica lemani*																			
		P2: 1	P2: 2	P2: 3	P2: 4	P2: 5	P2: 6	P2: 7	P2: 8	P2: 9	P2: 10	P2: 11	P2: 12	P2: 13	P2: 14	P2: 15	P2: 16	P2: 17	P2: 18	P2: 19	P2: 20
Abundance of M	Tricosene	598	4748	0	2420	1863		0	0	8063	2174	1172	6696	2583	892	7817	12007	7074	5764	8803	11407
	Tricosane	1267	2731	0	1407	2046		0	0	4110	1524	1190	5366	2425	547	3457	6155	3089	2124	8845	5596
	3 Me C23	3672	7584	0	7241	4776		1925	0	14610	7711	1932	14388	8493	3276	26544	34240	19176	13260	14506	34336
	Pentacosene	59400	145344	4777	97720	79400		37496	9200	219776	92200	61344	207680	105408	36944	274048	378432	212800	171904	206784	322944
	Pentacosane	3476	4084	2786	4440	3957		316	464	8521	5558	5425	8855	5789	956	7292	8435	11899	6569	31408	11035
	3 Me C25	14723	43848	2436	28112	37624		8980	721	71368	24528	13496	92592	31448	9488	94784	131072	84104	60760	62280	98784
	Heptacosene	23344	55608	908	43232	31184		12390	1572	87328	36232	25600	87080	43424	11812	107216	157120	92184	81680	78904	139456
	Heptacosane	844	1810	673	0	772		0	0	1861	1227	1674	1876	930	0	878	2189	2755	1906	9235	1221
Abundance of M+1	Tricosene	232	1784	0	816	854		0	0	1637	745	269	3106	543	257	2758	3400	2069	1198	1538	3361
	Tricosane	364	535	0	234	500		0	0	1006	557	219	1050	221	0	816	1293	742	1030	2296	937
	3 Me C23	1099	2674	0	1322	1182		570	0	4036	2388	558	3823	1203	225	5127	6965	3177	4710	2936	8165
	Pentacosene	16472	38288	1390	24664	19664		8692	1334	55488	20264	15979	58240	28648	9938	66624	97928	56648	43224	61768	84056
	Pentacosane	996	2339	1313	1209	1877		0	187	2013	2505	1898	4159	2067	361	1320	2382	4270	2006	10908	3018
	3 Me C25	4389	11615	531	8686	10132		2013	348	19872	7808	3621	18560	7373	2642	21952	33696	20120	17784	15122	24760
	Heptacosene	5613	18880	591	11231	8952		4129	1011	25248	10907	6790	25816	13125	3855	32504	40880	28144	21784	22928	33952
	Heptacosane	158	864	258	0	251		0	0	666	262	456	659	346	0	565	387	351	348	2508	489
Abundance of M+2	Tricosene	0	200	0	0	271		0	0	0	169	0	425	0	0	452	381	0	0	0	379
	Tricosane	0	0	0	0	165		0	0	0	267	0	0	0	0	199	0	159	0	0	220
	3 Me C23	0	459	0	831	0		0	0	165	301	366	453	0	0	670	565	482	1204	423	941
	Pentacosene	2800	5212	0	2760	3680		0	480	8325	2098	2885	7224	3624	593	9318	12927	7382	5829	5450	9423
	Pentacosane		417	0	0	326		0		289	0	762	321	0	0	153	220	739	0	628	378
	3 Me C25	0	1438	154	1307	1476		513	0	3263	1179	819	2287	1766	0	2358	3916	2041	2269	1393	3241
	Heptacosene	748	2080	0	1379	817		205	0	4186	1309	1119	0	1675	290	5653	5580	3914	2585	3945	6022
	Heptacosane	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	329	0	650	178
Percentage of M+1	Tricosene	38.8	37.6		33.7	45.8				20.3	34.3	23.0	46.4	21.0	28.8	35.3	28.3	29.2	20.8	17.5	29.5
	Tricosane	28.7	19.6		16.6	24.4		29.6		24.5	36.5	18.4	19.6	9.1	0.0	23.6	21.0	24.0	48.5	26.0	16.7
	3 Me C23	29.9	35.3		18.3	24.7		27.6		31.0	28.9	26.6	14.2	6.9	19.3	20.3	16.6	35.5	20.2	23.8	
	Pentacosene	27.7	26.3	29.1	25.2	24.8		23.2	14.5	25.2	22.0	26.0	28.0	27.2	26.9	24.3	25.9	26.6	25.1	29.9	26.0
	Pentacosane	28.7	57.3	47.1	27.2	47.4		0.0	40.3	23.6	45.1	35.0	47.0	35.7	37.8	18.1	28.2	35.9	30.5	34.7	27.3
	3 Me C25	29.8	26.5	21.8	30.9	26.9		22.4	48.3	27.8	31.8	26.8	20.0	23.4	27.8	23.2	25.7	23.9	29.3	24.3	25.1
	Heptacosene	24.0	34.0	65.1	26.0	28.7		33.3	64.3	28.9	30.1	26.5	29.6	30.2	32.6	30.3	26.0	30.5	26.7	29.1	24.3
	Heptacosane	18.7	47.7	38.3		32.5				35.8	21.4	27.2	35.1	37.2		64.4	17.7	12.7	18.3	27.2	40.0
Percentage of M+2	Tricosene	0.0	4.2		0.0	14.5				0.0	7.8	0.0	6.3	0.0	0.0	5.8	3.2	0.0	0.0	0.0	3.3
	Tricosane	0.0	0.0		0.0	8.1				0.0	17.5	0.0	0.0	0.0	0.0	5.8	0.0	5.1	0.0	0.0	3.9
	3 Me C23	0.0	6.1		11.5	0.0		0.0		1.1	3.9	18.9	3.1	0.0	0.0	2.5	1.7	2.5	9.1	2.9	2.7
	Pentacosene	4.7	3.6	0.0	2.8	4.6		0.0	5.2	3.8	2.3	4.7	3.5	3.4	1.6	3.4	3.4	3.5	3.4	2.6	2.9
	Pentacosane	0.0	10.2	0.0	0.0	8.2		0.0	0.0	3.4	0.0	14.0	3.6	0.0	0.0	2.1	2.6	6.2	0.0	2.0	3.4
	3 Me C25	0.0	3.3	6.3	4.6	3.9		5.7	0.0	4.6	4.8	6.1	2.5	5.6	0.0	2.5	3.0	2.4	3.7	2.2	3.3
	Heptacosene	3.2	3.7	0.0	3.2	2.6		1.7	0.0	4.8	3.6	4.4	0.0	3.9	2.5	5.3	3.6	4.2	3.2	5.0	4.3
	Heptacosane	0.0	0.0	0.0		0.0				0.0	0.0	0.0	0.0	0.0		0.0	0.0	11.9	0.0	7.0	14.6

0
1-20
20-40
40-60
60-80
80-100
100+

Figure B.16: Data sheet showing the raw propionic-2,2-d_2 acid-d data for the preliminary propionate experiment for *Formica lemani*.

Propionic Acid (Control)		*Myrmica sabuleti*																			
		Con 1	Con 2	Con 3	Con 4	Con 5	Con 6	Con 7	Con 8	Con 9	Con 10	Con 11	Con 12	Con 13	Con 14	Con 15	Con 16	Con 17	Con 18	Con 19	Con 20
Abundance of M	Tricosane	7282	8459	10884	8679	15112	14599	14595	35320	48160	18568	46368	42832	14592	17488	19896	20288	11534	32912	31752	27720
	5 Me C23	11646	12799	11007	15838	34544	23896	10291	27960	77232	20656	44672	17840	14286	26008	23056	11181	14846	35872	76872	59184
	Pentacosene	51512	44912	53480	69656	104256	83680	43920	87992	217536	73896	138048	79104	57600	91896	76288	42392	62328	105488	248896	203392
	Pentacosane	6442	3428	5516	6636	8518	5390	10738	19848	28280	12197	25672	21728	6953	9284	9614	11616	7075	22992	14713	11944
	11 13 Me C25	21416	18880	27056	16880	51688	35656	17872	33904	98216	34328	55440	24424	19048	28208	32792	27128	33256	58096	90536	69048
	5 Me C25	71560	72240	84824	88784	180032	139904	79480	181248	398208	146304	251328	138624	94728	143424	139328	86560	140224	278144	301312	292800
	Heptacosene	10887	9538	10598	13117	23488	12773	7499	17240	48512	17024	32000	12818	10059	17344	14435	8349	15160	33072	49104	39240
	5 Me C27	9080	12992	21800	16090	40696	28176	15041	41112	103768	40424	64032	31232	18344	30720	27560	26344	35904	90632	72128	65664
Abundance of M+1	Tricosane	1532	1600	3887	2993	4673	4026	2654	7943	12003	4922	13373	11952	4078	4403	5221	5390	3955	9796	9929	6929
	5 Me C23	2246	1836	2378	2785	8025	5551	2052	7122	18552	3969	10486	3437	2728	4251	4574	2678	3711	6973	13248	13615
	Pentacosene	12889	12160	14632	15733	25896	25376	11810	24432	60208	21416	38576	23848	13852	22296	23256	10960	16275	27616	65200	49552
	Pentacosane	1023	1279	1132	646	1983	1862	5025	3065	6114	2530	6106	6536	1688	3169	2931	3370	2561	6167	3989	3446
	11 13 Me C25	10924	10252	17440	11725	25536	21952	8501	16696	54584	18872	32920	13195	8046	13783	15872	12458	21760	33064	47128	37976
	5 Me C25	17912	17128	17512	18664	42008	31112	16050	44960	87384	32536	55424	27984	19544	29280	30440	18040	30664	58744	60408	61896
	Heptacosene	3238	2460	4366	3504	6419	3599	2149	6131	15358	4735	9291	5573	3208	4643	3499	2303	3681	10338	15621	11173
	5 Me C27	2356	3669	5756	3862	7599	6803	1988	7617	23008	8682	14694	8841	5336	7598	6672	5150	7383	21680	16456	14102
Abundance of M+2	Tricosane	743	0	686	210	447	169	158	566	2576	1145	2121	1817	650	184	557	866	717	926	893	1058
	5 Me C23	0	447	273	171	786	657	444	454	2171	730	941	705	509	607	1058	412	181	537	1350	776
	Pentacosene	3190	2174	1728	1977	3683	3801	2602	2694	6716	3512	5088	2471	3513	2636	2761	1960	3032	3197	7669	6202
	Pentacosane	205	0	0	0	819	165	348	869	908	224	895	1191	0	1062	586	629	188	819	432	0
	11 13 Me C25	1312	1337	1747	1558	3314	1918	1272	1687	5760	2596	6192	1939	2005	2104	2951	1452	1727	4535	5435	4913
	5 Me C25	2121	2040	1016	1985	5122	4389	1320	4250	8603	3119	4985	3183	3071	3672	3369	1711	3400	6921	6102	5304
	Heptacosene	976	243	157	466	838	792	204	652	2506	465	1593	411	627	1313	1627	318	1069	1199	1513	2205
	5 Me C27	365	215	936	429	926	510	330	1690	3121	701	1489	1181	794	954	599	829	1575	2901	2594	2524
Percentage of M+1	Tricosane	21.0	18.9	35.7	34.5	30.9	27.6	18.2	22.5	24.9	26.5	28.8	27.9	27.9	25.2	26.2	26.6	34.3	29.8	31.3	25.0
	5 Me C23	19.3	14.3	21.6	17.6	23.2	23.2	19.9	25.5	24.0	19.2	23.5	19.3	19.1	16.3	19.8	24.0	25.0	19.4	17.2	23.0
	Pentacosene	25.0	27.1	27.4	22.6	24.8	30.3	26.9	27.8	27.7	29.0	27.9	30.1	24.0	24.3	30.5	25.9	26.1	26.2	26.2	24.4
	Pentacosane	15.9	37.3	20.5	9.7	23.3	34.5	46.8	15.4	21.6	20.7	23.8	30.1	24.3	34.1	30.5	29.0	36.2	26.8	27.1	28.9
	11 13 Me C25	51.0	54.3	64.5	69.5	49.4	61.6	47.6	49.2	55.6	55.0	59.4	54.0	42.2	48.9	48.4	45.9	65.4	56.9	52.1	55.0
	5 Me C25	25.0	23.7	20.6	21.0	23.3	22.2	20.2	24.8	21.9	22.2	22.1	20.2	20.6	20.4	21.8	20.8	21.9	21.1	20.0	21.1
	Heptacosene	29.7	25.8	41.2	26.7	27.3	28.2	28.7	35.6	31.7	27.8	29.0	43.5	31.9	26.8	24.2	27.6	24.3	31.3	31.8	28.5
	5 Me C27	25.9	28.2	26.4	24.0	18.7	24.1	13.2	18.5	22.2	21.5	22.9	28.3	29.1	24.7	24.2	19.5	20.6	23.9	22.8	21.5
Percentage of M+2	Tricosane	10.2	0.0	6.3	2.4	3.0	1.2	1.1	1.6	5.3	6.2	4.6	4.2	4.5	1.1	2.8	4.3	6.2	2.8	2.8	3.8
	5 Me C23	0.0	3.5	2.5	1.1	2.3	2.7	4.3	1.6	2.8	3.5	2.1	4.0	3.6	2.3	4.6	3.7	1.2	1.5	1.8	1.3
	Pentacosene	6.2	4.8	3.2	2.8	3.5	4.5	5.9	3.1	3.1	4.8	3.7	3.1	6.1	2.9	3.6	4.6	4.9	3.0	3.1	3.0
	Pentacosane	3.2	0.0	0.0	0.0	9.6	3.1	3.2	4.4	3.2	1.8	3.5	5.5	0.0	11.4	6.1	5.4	2.7	3.6	2.9	0.0
	11 13 Me C25	6.1	7.1	6.5	9.2	6.4	5.4	7.1	5.0	5.9	7.6	11.2	7.9	10.5	7.5	9.0	5.4	5.2	7.8	6.0	7.1
	5 Me C25	3.0	2.8	1.2	2.2	2.8	3.1	1.7	2.3	2.2	2.1	2.0	2.3	3.2	2.6	2.4	2.0	2.4	2.5	2.0	1.8
	Heptacosene	9.0	2.5	1.5	3.6	3.6	6.2	2.7	3.8	5.2	2.7	5.0	3.2	6.2	7.6	11.3	3.8	7.1	3.6	3.1	5.6
	5 Me C27	4.0	1.7	4.3	2.7	2.3	1.8	2.2	4.1	3.0	1.7	2.3	3.8	4.3	3.1	2.2	3.1	4.4	3.2	3.6	3.8

0
1-20
20-40
40-60
60-80
80-100
100+

Figure B.17: Data sheet showing the raw control data for the preliminary propionate experiment for *Myrmica sabuleti*.

Sodium [1-^{13}C]propionate		*Myrmica sabuleti*																							
		P1: 1	P1: 2	P1: 3	P1: 4	P1: 5	P1: 6	P1: 7	P1: 8	P1: 9	P1: 10	P1: 11	P1: 12	P1: 13	P1: 14	P1: 15	P1: 16	P1: 17	P1: 18	P1: 19	P1: 20	P1: 21	P1: 22	P1: 23	P1: 24
Abundance of M	Tricosane	19992	26568	13088	26896	16904	20432	20152	22784	16308	30728	26528	16924	16736	16261	18824	32320	43048	44264	42392	40768	33896	27280	51776	57528
	5 Me C23	23488	52400	15667	49048	18512	35504	16880	16840	15060	41416	29392	12531	23480	7247	22504	41800	70760	84704	46592	60024	31168	36096	39520	38856
	Pentacosene	120608	208192	91272	222784	97344	211200	66496	82224	66456	193280	119408	80288	130344	45864	127176	170624	258944	343552	198080	269568	137088	103976	139584	158080
	Pentacosane	4973	9267	4401	9701	4094	8515	9779	7792	6781	14888	11720	7597	8095	7895	8422	15422	13113	14603	22304	16183	16171	12302	24344	21464
	11 13 Me C25	31280	56008	24360	60008	18616	54584	20384	20144	15035	50344	32376	18192	37944	9971	28008	43312	62504	91656	48728	78544	34272	26744	39816	39112
	5 Me C25	112320	236480	75856	207808	89536	185024	106048	95000	96424	232384	153920	93400	130888	64664	116968	220544	277248	334912	254656	287744	168384	166464	200192	190848
	Heptacosene	21104	45000	17112	45408	17752	41506	12510	15478	13152	43624	26688	15701	21776	6988	23384	35624	50824	66072	43736	57320	24056	17032	27608	37656
	5 Me C27	21024	45448	18032	44184	15636	37936	19712	14755	17272	49312	41128	18608	27688	13617	22424	40848	66496	62712	64120	59896	33928	29928	44328	44736
Abundance of M+1	Tricosane	3957	5818	4569	7125	4487	5936	5122	4403	4078	8428	6896	2954	5643	3992	6382	8377	9750	10328	11360	8970	8219	8112	10901	13971
	5 Me C23	15264	31768	10005	19936	11683	20408	11300	10732	10187	22880	16208	9377	14222	5100	15318	29600	43440	35200	38264	34840	20416	23480	26920	28360
	Pentacosene	34296	53528	26072	61200	24856	55360	16992	21360	18576	48992	30688	18088	39256	13496	30712	47744	62464	92288	53600	79472	35896	25000	39136	46376
	Pentacosane	1447	2308	1491	3303	1589	2814	2282	2133	1544	3450	4550	2119	1214	2572	1925	4135	3483	3610	4785	5327	3461	3803	7395	4030
	11 13 Me C25	24024	50032	24488	53736	19776	34464	29288	19200	15091	45592	35712	28664	34800	12671	24960	47968	63912	73488	60000	64040	41232	25864	43864	45864
	5 Me C25	76464	195328	68032	117120	78320	92968	106112	83352	113680	164288	129104	110136	79184	78600	105792	212160	230400	170944	298752	173760	162048	182144	188160	188160
	Heptacosene	6325	11693	6864	11925	5402	12819	5365	6320	4768	12699	7577	5382	8986	1695	8114	10180	13271	20928	12365	18416	8678	6648	9571	10855
	5 Me C27	14314	36944	12247	31128	14512	27744	23080	15990	18056	43912	33248	22552	21080	20008	24840	49928	57728	38640	76808	48592	30928	36752	46136	53928
Abundance of M+2	Tricosane	980	1117	643	840	801	762	835	886	0	497	956	390	570	462	383	951	1418	1229	1459	1294	909	1394	1894	1273
	5 Me C23	1714	7787	1574	3750	1833	3508	1782	2539	2876	3855	3484	1438	1347	1423	3567	6206	7351	5832	7879	5282	4272	4995	4047	4262
	Pentacosene	3935	7564	3440	7040	3202	7788	3636	2842	2171	5153	2974	3126	4420	1679	3560	6262	9749	9271	6942	8822	6773	2112	5575	5580
	Pentacosane	475	285	479	873	366	626	520	0	533	796	0	481	264	739	314	434	549	226	764	592	587	290	423	859
	11 13 Me C25	6345	16616	6245	14310	5248	8873	8191	6351	9794	14130	11444	9867	8933	5468	7296	16296	20096	15402	23144	19048	12300	8507	16165	13265
	5 Me C25	14655	34256	14030	21312	15931	17320	22808	18696	22600	33040	24200	22808	13335	16416	20800	44920	45528	26008	57672	32064	30936	38648	40520	41504
	Heptacosene	973	1021	277	2059	1036	1640	255	615	157	2141	421	1176	1778	670	849	1400	1490	3028	2438	2608	1093	934	1610	1216
	5 Me C27	3941	9026	2169	4377	3710	5305	7333	3417	4698	9156	7631	5375	4216	3832	4550	10833	12430	7808	18800	10252	6055	7463	11820	12299
Percentage of M+1	Tricosane	19.8	21.9	34.9	26.5	26.5	29.1	25.4	19.3	25.0	27.4	26.0	17.5	33.7	24.5	33.9	25.9	22.6	23.3	26.8	22.0	24.2	29.7	21.1	24.3
	5 Me C23	65.0	60.6	63.9	40.6	63.1	57.5	66.9	63.7	67.6	55.2	55.1	74.8	60.6	70.4	68.1	70.8	61.4	41.6	82.1	58.0	65.5	65.0	68.1	73.0
	Pentacosene	28.4	25.7	28.6	27.5	25.5	26.2	25.6	26.0	28.0	25.3	25.7	22.5	30.1	29.4	24.1	28.0	24.1	26.9	27.1	29.5	26.2	24.0	28.0	29.3
	Pentacosane	29.1	24.9	33.9	34.0	38.8	33.0	23.3	27.4	22.8	23.2	38.8	27.9	15.0	32.6	22.9	26.8	26.6	24.7	21.5	32.9	21.4	30.9	30.4	18.8
	11 13 Me C25	76.8	89.3	100.5	89.5	106.2	63.1	143.7	95.3	100.4	90.6	110.3	157.6	91.7	127.1	89.1	110.7	102.3	80.2	123.1	81.5	120.3	96.7	110.2	117.3
	5 Me C25	68.1	82.6	89.7	56.4	87.5	50.2	100.1	87.7	117.9	70.7	83.9	117.9	60.5	121.6	90.4	96.2	83.1	51.0	117.3	60.4	96.2	109.4	94.0	98.6
	Heptacosene	30.0	26.0	40.1	26.3	30.4	30.9	42.9	40.8	36.3	29.1	28.4	34.3	41.3	24.3	34.7	28.6	26.1	31.7	28.3	32.1	36.1	39.0	34.7	28.8
	5 Me C27	68.1	81.3	67.9	70.5	92.8	73.1	117.1	108.4	104.5	89.0	80.8	121.2	76.1	146.9	110.8	122.2	86.8	61.6	119.8	81.1	91.2	122.8	104.1	120.5
Percentage of M+2	Tricosane	4.9	4.2	4.9	3.1	4.7	3.7	4.1	3.9	0.0	1.6	3.6	2.3	3.4	2.8	2.0	2.9	3.3	2.8	3.4	3.2	2.7	5.1	3.7	2.2
	5 Me C23	7.3	14.9	10.0	7.6	9.9	9.9	10.6	15.1	19.1	9.3	11.9	11.5	5.7	19.6	15.9	14.8	10.4	6.9	16.9	8.8	13.7	13.8	10.2	11.0
	Pentacosene	3.3	3.6	3.8	3.2	3.3	3.7	5.5	3.5	3.3	2.7	2.5	3.9	3.4	3.7	2.8	3.7	3.8	2.7	3.5	3.3	4.9	2.0	4.0	3.5
	Pentacosane	9.6	3.1	10.9	9.0	8.9	7.4	5.3	0.0	7.9	5.3	0.0	6.3	3.3	9.4	3.7	2.8	4.2	1.5	3.4	3.7	3.6	2.4	1.7	4.0
	11 13 Me C25	20.3	29.7	25.6	23.8	28.2	16.3	40.2	31.5	65.1	28.1	35.3	54.2	23.5	54.8	26.0	37.6	32.2	16.8	47.5	24.3	35.9	31.8	40.6	33.9
	5 Me C25	13.0	14.5	18.5	10.3	17.8	9.4	21.5	19.7	23.4	14.2	15.7	24.4	10.2	25.4	17.8	20.4	16.4	7.8	22.6	11.1	18.4	23.2	20.2	21.7
	Heptacosene	4.6	2.3	1.6	4.5	5.8	4.0	2.0	4.0	1.2	4.9	1.6	7.5	8.2	9.6	3.6	3.9	2.9	4.6	5.6	4.5	4.5	5.5	5.8	3.2
	5 Me C27	18.7	19.9	12.0	9.9	23.7	14.0	37.2	23.2	27.2	18.6	18.6	28.9	15.2	28.1	20.3	26.5	18.7	12.5	29.3	17.1	17.8	24.9	26.7	27.5

0
0-20
20-40
40-60
60-80
80-100
100+

Figure B.18: Data sheet showing the raw sodium [1-^{13}C]propionate data for the preliminary propionate experiment for *Myrmica sabuleti*.

Propionic-2,2-d_2 acid-d		*Myrmica sabuleti*																				
		P2: 1	P2: 2	P2: 3	P2: 4	P2: 5	P2: 6	P2: 7	P2: 8	P2: 9	P2: 10	P2: 11	P2: 12	P2: 13	P2: 14	P2: 15	P2: 16	P2: 17	P2: 18	P2: 19	P2: 20	P2: 21
Abundance of M	Tricosane	17640	38200	50928	11628	15918	24312	24472	24560	24360	32264	27168	41224	39360	67744	14727	53736	32504	43344	19400	30952	56400
	5 Me C23	46768	63200	60208	16179	29152	31664	27040	30160	27576	38216	54880	65448	79888	85488	25912	51432	23840	48712	22232	44944	60960
	Pentacosene	192640	219264	207872	67336	127048	126080	115264	111008	126592	188160	257472	272448	306944	339584	93104	210240	110272	195456	106032	201472	221568
	Pentacosane	6731	10522	21408	3095	5105	7981	9084	13017	10643	10193	7017	19768	13643	27216	6100	23712	12320	15622	6533	8948	24088
	11 13 Me C25	71256	87296	79880	18688	43136	42088	37992	38000	36304	60296	72144	81000	88904	104936	31368	78048	39016	64072	33608	62792	62480
	5 Me C25	243998	297472	297600	80400	133440	151936	160192	148224	143296	206592	260803	300416	300032	400003	132608	293056	163584	45824	153344	221312	337792
	Heptacosene	43688	48840	46656	11099	20848	24976	21296	18184	23240	36592	44080	49704	57624	66296	14399	45112	19304	49856	18344	38104	41808
	5 Me C27	50416	66224	70024	16600	24048	29768	29752	30568	30384	49512	50368	53568	70976	75360	20544	70648	27320	60584	27136	33680	62176
Abundance of M+1	Tricosane	3986	8935	13614	3968	3996	7032	6800	5716	5868	6643	7291	9752	9531	12304	3593	16110	9353	11526	6101	8584	14931
	5 Me C23	11185	17696	17512	4177	6959	8898	5991	8706	8111	10436	13472	17104	19304	23320	7663	14292	6228	10936	5503	12443	15142
	Pentacosene	58152	56368	58128	17056	34288	38808	33672	26360	35544	50232	68696	72616	83888	88920	23312	58176	32704	52224	24664	45472	59224
	Pentacosane	1396	3579	4672	808	1319	1951	2636	2850	3138	3849	3669	5885	4462	6541	1820	6277	3674	4730	2451	2218	8497
	11 13 Me C25	43864	51728	54984	10516	26664	25896	21640	24136	22528	34696	44336	47080	57216	63952	16848	45736	21512	35352	23208	33688	38936
	5 Me C25	65520	95640	95304	26816	40680	54912	45712	53112	41488	68224	69176	99840	94600	114944	42832	94680	52512	13161	50440	70064	98696
	Heptacosene	13915	15151	13168	3741	9387	7733	7039	6582	7200	9516	12712	13678	20576	22984	4837	15544	6669	17584	5850	11777	13267
	5 Me C27	15631	21288	25768	5644	7796	11580	10962	10281	8238	15235	15222	17840	22480	26184	5986	22368	11823	18176	8210	12282	20344
Abundance of M+2	Tricosane	912	1351	3043	253	547	804	976	1185	608	1283	1275	1101	1487	1214	395	1297	1124	2103	1140	1023	3060
	5 Me C23	1465	3072	2528	1117	905	1213	413	1114	1699	2373	2246	3156	2071	2839	1012	1962	1418	2689	972	1987	3501
	Pentacosene	6064	8003	7721	3436	5633	4328	4892	2716	4507	5161	8840	8227	9785	10048	3304	7969	4780	7864	4073	6518	6686
	Pentacosane	177	635	1251	0	236	202	700	808	496	797	317	686	0	1457	404	678	382	531	364	431	722
	11 13 Me C25	6425	8095	9696	1599	3447	4128	2552	2982	3582	4589	4364	7438	6450	12192	2920	5114	2915	6388	4117	5089	6253
	5 Me C25	10325	14215	14736	4069	5195	8051	8527	5446	7491	7626	9036	13727	12517	20008	6295	14939	6101	1827	7565	9751	12882
	Heptacosene	1323	1949	2218	336	1509	2062	542	829	1762	1344	2374	1940	2465	3162	908	1604	1258	3179	1230	1955	1826
	5 Me C27	2850	3654	3975	180	545	1520	783	2527	2388	2316	1480	2677	3404	4047	1459	3788	1768	3346	1648	2343	3453
Percentage of M+1	Tricosane	22.6	23.4	26.7	34.1	25.1	28.9	27.8	23.3	24.1	20.6	26.8	23.7	24.2	18.2	24.4	30.0	28.8	26.6	31.4	27.7	26.5
	5 Me C23	23.9	28.0	29.1	25.8	23.9	28.1	22.2	28.9	29.4	27.3	24.5	26.1	24.2	27.3	29.6	27.8	26.1	22.5	24.8	27.7	24.8
	Pentacosene	30.2	25.7	28.0	25.3	27.0	30.8	29.2	23.7	28.1	26.7	26.7	26.7	27.3	26.2	25.0	27.7	29.7	26.7	23.3	22.6	26.7
	Pentacosane	20.7	34.0	21.8	26.1	25.8	24.4	29.0	21.9	29.5	37.8	52.3	29.8	32.7	24.0	29.8	26.5	29.8	30.3	37.5	24.8	35.3
	11 13 Me C25	61.6	59.3	68.8	56.3	61.8	61.5	57.0	63.5	62.1	57.5	61.5	58.1	64.4	60.9	53.7	58.6	55.1	55.2	69.1	53.7	62.3
	5 Me C25	27.3	32.2	32.0	33.4	30.5	36.1	28.5	35.8	29.0	33.0	26.5	33.2	31.5	28.7	32.3	32.3	32.1	28.7	32.9	31.7	29.2
	Heptacosene	31.9	31.0	28.2	33.7	45.0	31.0	33.1	36.2	31.0	26.0	28.8	27.5	35.7	34.7	33.6	34.5	34.5	35.3	31.9	30.9	31.7
	5 Me C27	31.0	32.1	36.8	34.0	32.4	38.9	36.8	33.6	27.1	30.8	30.2	33.3	31.7	34.7	29.1	31.7	43.3	30.0	30.3	36.5	32.7
Percentage of M+2	Tricosane	5.2	3.5	6.0	2.2	3.4	3.3	4.0	4.8	2.5	4.0	4.7	2.7	3.8	1.8	2.7	2.4	3.5	4.9	5.9	3.3	5.4
	5 Me C23	3.1	4.9	4.2	6.9	3.1	3.8	1.5	3.7	6.2	6.2	4.1	4.8	2.6	3.3	3.9	3.8	5.9	5.5	4.4	4.4	5.7
	Pentacosene	3.1	3.6	3.7	5.1	4.4	3.4	4.2	2.4	3.6	2.7	3.4	3.0	3.2	3.0	3.5	3.8	4.3	4.0	3.8	3.2	3.0
	Pentacosane	2.6	6.0	5.8	0.0	4.6	2.5	7.7	6.2	4.7	7.8	4.5	3.5	0.0	5.4	6.6	2.9	3.1	3.4	5.6	4.8	3.0
	11 13 Me C25	9.0	9.3	12.1	8.6	8.0	9.8	6.7	7.8	9.9	7.6	6.0	9.2	7.3	11.6	9.3	6.6	7.5	10.0	12.3	8.1	10.0
	5 Me C25	4.3	4.8	5.0	5.1	3.9	5.3	5.3	3.7	5.2	3.7	3.5	4.6	4.2	5.0	4.7	5.1	3.7	4.0	4.9	4.4	3.8
	Heptacosene	3.0	4.0	4.8	3.0	7.2	8.3	2.5	4.6	7.6	3.7	5.4	3.9	4.3	4.8	6.3	3.6	6.5	6.4	6.7	5.1	4.4
	5 Me C27	5.7	5.5	5.7	1.1	2.3	5.1	2.6	8.3	7.9	4.7	2.9	5.0	4.8	5.4	7.1	5.4	6.5	5.5	6.1	7.0	5.6

0
0-20
20-40
40-60
60-80
80-100
100+

Figure B.19: Data sheet showing the raw propionic-2,2-d_2 acid-d data for the preliminary propionate experiment for *Myrmica sabuleti*.

Propionic Acid (Negative Control)		*Myrmica sabuleti*																								
		Con 1	Con 2	Con 3	Con 4	Con 5	Con 6	Con 7	Con 8	Con 9	Con 10	Con 11	Con 12	Con 13	Con 14	Con 15	Con 16	Con 17	Con 18	Con 19	Con 20	Con 21	Con 22	Con 23	Con 24	Con 25
Abundance of M	Tricosane	18320	5452	13144	16210	3590	26784	17752	16440	8344	6586	9556	5702	15764	20120	36288	23688	22544	8851	46960	20336	31496	22144	11083	30224	21008
	5 Me C23	45152	15229	32928	44024	21464	55944	44168	46008	23928	14660	27368	14350	36920	46592	94968	62392	43008	20296	100800	60648	46872	61016	33272	92536	65736
	Pentacosene	274688	109008	173504	190528	121752	269696	266688	181696	111632	81800	169408	116768	256896	220800	462464	269376	252416	124896	407488	311168	230208	318080	149824	318208	299456
	11 13 Me C25	59160	14129	37160	35912	16832	84152	44792	44280	3419	8791	31776	13895	53496	36752	97808	55304	46896	12432	105680	70768	34648	59592	14883	66864	66840
	5 Me C25	281280	153344	167936	289600	159424	320192	335360	227840	165120	131264	187840	108264	241088	263360	524864	359040	250240	222592	476096	340672	413760	354816	273984	451136	313664
	Heptacosene	24992	8482	12058	18416	7283	41272	27392	22632	8051	5660	12461	3538	20576	19776	59320	20936	22208	10156	52936	32032	24048	33944	12882	36008	23216
Abundance of M+1	Tricosane	3663	1197	2034	5097	1222	6409	5622	5357	3044	2283	1836	2396	2129	6606	8153	5571	3156	2993	15887	4127	8126	5185	4100	5118	4829
	5 Me C23	9623	3505	7363	6290	4682	10516	10268	9741	5034	3216	4474	1189	8871	9711	18896	14753	8898	5275	21504	11453	11121	15595	4836	11592	18104
	Pentacosene	79400	27352	45488	47480	30648	64896	76104	46272	32112	26152	40936	24800	70144	63896	122856	76464	65344	41184	110424	80340	74752	86128	36952	85528	80944
	11 13 Me C25	34728	8567	20736	23472	10427	46720	28008	25680	1239	7165	16992	6360	27064	19512	56640	32672	30696	9150	62792	29256	25632	36992	11126	35576	34792
	5 Me C25	64384	27120	41472	59680	35384	80680	68616	52488	37344	24984	36120	23536	57016	59880	124720	75440	57296	55856	110616	80944	92240	85152	58120	97160	71888
	Heptacosene	6850	1395	5190	7431	2845	9686	7098	5745	1959	1711	2989	1609	6063	5387	13780	8186	5842	3016	16237	11923	6602	7722	4650	14581	8149
Abundance of M+2	Tricosane	1092	333	723	169	380	278	215	986		231			332	447	2438	611			840	975	1275	2941		592	2134
	5 Me C23	868		442		942		2116	1101	980	455			860	737	952	1019	1648	411	2820	1077			1751	3632	1165
	Pentacosene	10147	2634	9032	7490	6611	5717	11286	6149	4817	3320	5135	3337	7598	11687	15218	11125	6739	3478	12398	8797	7530	12304	4940	7991	11580
	11 13 Me C25	4410	879	2697	1633	1256	5303	3963	3892		697	1721	879	3127	1835	6500	3920	2361	1240	8987	4949	2756	4434	632	5086	5886
	5 Me C25	5117	3770	5056	4729	5164	7630	7647	6630	7161	3548	4292	2815	5650	7233	13425	7523	11407	4517	10534	6828	10032	7480	4134	10491	9663
	Heptacosene	647	426	875	720		1906	459	710	1095		405		1048	536	1788	940	1428	153	1484	800	797	636	516	3950	441
Percentage of M+1	Tricosane	20.0	22.0	15.5	31.4	34.0	23.9	31.7	32.6	36.5	34.7	19.2	42.0	13.5	32.8	22.5	23.5	14.0	33.8	33.8	20.3	25.8	23.4	37.0	16.9	23.0
	5 Me C23	21.3	23.0	22.4	14.3	21.8	18.8	23.2	21.2	21.0	21.9	16.3	8.3	24.0	20.8	19.9	23.6	20.7	26.0	21.3	18.9	23.7	25.6	14.5	12.5	27.5
	Pentacosene	28.9	25.1	26.2	24.9	25.2	24.1	28.5	25.5	28.8	32.0	24.2	21.2	27.3	28.9	26.6	28.4	25.9	33.0	27.1	25.8	32.5	27.1	24.7	26.9	27.0
	11 13 Me C25	58.7	60.6	55.8	65.4	61.9	55.5	62.5	58.0	36.2	81.5	53.5	45.8	50.6	53.1	57.9	59.1	65.5	73.6	59.4	41.3	74.0	62.1	74.8	53.2	52.1
	5 Me C25	22.9	17.7	24.7	20.6	22.2	25.2	20.5	23.0	22.6	19.0	19.2	21.7	23.6	22.7	23.8	21.0	22.9	25.1	23.2	23.8	22.3	24.0	21.2	21.5	22.9
	Heptacosene	27.4	16.4	43.0	40.4	39.1	23.5	25.9	25.4	24.3	30.2	24.0	45.5	29.5	27.2	23.2	39.1	26.3	29.7	30.7	37.2	27.5	22.7	36.1	40.5	35.1
Percentage of M+2	Tricosane	6.0	6.1	5.5	1.0	10.6	1.0	1.2	6.0	0.0	3.5	0.0	0.0	2.1	2.2	6.7	2.6	0.0	0.0	1.8	4.8	4.0	13.3	0.0	2.0	10.2
	5 Me C23	1.9	0.0	1.3	0.0	4.4	0.0	4.8	2.4	4.1	3.1	0.0	0.0	2.3	1.6	1.0	1.6	3.8	2.0	2.8	1.8	0.0	0.0	5.3	3.9	1.8
	Pentacosene	3.7	2.4	5.2	3.9	5.4	2.1	4.2	3.4	4.3	4.1	3.0	2.9	3.0	5.3	3.3	4.1	2.7	2.8	3.0	2.8	3.3	3.9	3.3	2.5	3.9
	11 13 Me C25	7.5	6.2	7.3	4.5	7.5	6.3	8.8	8.8	0.0	7.9	5.4	6.3	5.8	5.0	6.6	7.1	5.0	10.0	8.5	7.0	8.0	7.4	4.2	7.6	8.8
	5 Me C25	1.8	2.5	3.0	1.6	3.2	2.4	2.3	2.9	4.3	2.7	2.3	2.6	2.3	2.7	2.6	2.1	4.6	2.0	2.2	2.0	2.4	2.1	1.5	2.3	3.1
	Heptacosene	2.6	5.0	7.3	3.9	0.0	4.6	1.7	3.1	13.6	0.0	3.3	0.0	5.1	2.7	3.0	4.5	6.4	1.5	2.8	2.5	3.3	1.9	4.0	11.0	1.9

0
0-20
20-40
40-60
60-80
80-100
100+

Figure B.20: Data sheet showing page one of the raw negative control data (propionic acid) for the propionate experiment for *Myrmica sabuleti*.

Propionic Acid (Negative Control)		*Myrmica sabuleti*																								
		Con 26	Con 27	Con 28	Con 29	Con 30	Con 31	Con 32	Con 33	Con 34	Con 35	Con 36	Con 37	Con 38	Con 39	Con 40	Con 41	Con 42	Con 43	Con 44	Con 45	Con 46	Con 47	Con 48	Con 49	Con 50
Abundance of M	Tricosane	2718	1918	13632	10762	2312	5189	4638	6172	3109	872	1792	1957	1842	1715	1014	3875	1444	4049	1763	1075	464	1282	1722	2694	3172
	5 Me C23	6991	6664	21472	21488	5221	10789	11565	11625	10201	6622	4158	7953	3139	3542	3693	3375	9083	7595	6890	4792	2704	5353	3494	7258	7288
	Pentacosene	31024	54328	176448	153408	44184	47064	76856	66376	66520	18856	22960	36440	16920	16299	26288	37424	26344	48576	20264	24872	15867	22080	17832	42168	35120
	11 13 Me C25	4474	1906	21960	21856	10156	8590	18792	16027	9446	20312	4827	7722	4261	1469	1165	7018	6629	6452	4394	1548	3199	6067	3988	1041	8841
	5 Me C25	36568	53664	186688	168832	49848	57880	77680	69400	86008		24472	40972	24752	17112	32456	45288	38568	50032	48056	32336	12588	24960	10301	42256	32592
	Heptacosene	1780	2179	15287	13604	2193	3452	7784	4878	6285		1701	2329	1127		3745	1411	1886	3533	1186	1403	1580	2822	1999	3110	4090
Abundance of M+1	Tricosane	160	472	3790	2817	928	968	2361	309	1161	394	285	252	283	377	275	673	528	944	658	331		211	242	500	519
	5 Me C23	1499	2371	4440	4831	1036	1549	2592	2677	3181	1900	1892	2029	514	1371	290	1100	1390	2019	1763	1421	696	1081	739	1392	2153
	Pentacosene	10415	15369	43440	36088	9797	12254	26728	14808	18944	6934	6437	8717	6866	4236	6382	11627	8503	12969	6227	6198	3937	6828	4739	8654	10572
	11 13 Me C25	1165	323	18024	12025	3284	6671	13299	10363	5606	2354	1986	5357	1770	569	852	6298	4861	3172	2280	432	1806	2856	3093	847	8069
	5 Me C25	8754	14120	40160	33048	7864	11049	18896	17616	20056	3166	7545	11015	3965	2983	826	7953	6894	11567	9406	7079	4158	5468	2343	10212	8601
	Heptacosene	877	1130	4923	3832	1409	1511	3335	1086	1899		255	1508	502		1562	571	506	1323	470	450	177	482	342	1615	851
Abundance of M+2	Tricosane				556								178													
	5 Me C23		1780	1173	1103		644	2375	196	217		311				253			171	311	352				1307	185
	Pentacosene	3106	2744	7721	5658	1919	1562	2799	2684	2451	859	917	871	757	928	1298	1499	820		526	825	1392	602	710	1487	1921
	11 13 Me C25	837		3355	2285	538	1638	1704	2013	500		285	805	425		689	860	240	554	701	157	633	303			1067
	5 Me C25	1853	1941	6784	3026	2216	773	1400	2665	1738	1032		1353	782	209		850	969	1131	1196	364	489	703		2025	875
	Heptacosene			516	178			556	250	306						266							277		228	
Percentage of M+1	Tricosane	5.9	24.6	27.8	26.2	40.1	18.7	50.9	5.0	37.3	45.2	15.9	12.9	15.4	22.0	27.1	17.4	36.6	23.3	37.3	30.8	0.0	16.5	14.1	18.6	16.4
	5 Me C23	21.4	35.6	20.7	22.5	19.8	14.4	22.4	23.0	31.2	28.7	45.5	25.5	16.4	38.7	7.9	32.6	15.3	26.6	25.6	29.7	25.7	20.2	21.2	19.2	29.5
	Pentacosene	33.6	28.3	24.6	23.5	22.2	26.0	34.8	22.3	28.5	36.8	28.0	23.9	40.6	26.0	24.3	31.1	32.3	26.7	30.7	24.9	24.8	30.9	26.6	20.5	30.1
	11 13 Me C25	26.0	16.9	82.1	55.0	32.3	77.7	70.8	64.7	59.3	11.6	41.1	69.4	41.5	38.7	73.1	89.7	73.3	49.2	51.9	27.9	56.5	47.1	77.6	81.4	91.3
	5 Me C25	23.9	26.3	21.5	19.6	15.8	19.1	24.3	25.4	23.3		30.8	26.9	16.0	17.4	2.5	17.6	17.9	23.1	19.6	21.9	33.0	21.9	22.7	24.2	26.4
	Heptacosene	49.3	51.9	32.2	28.2	64.2	43.8	42.8	22.3	30.2		15.0	64.7	44.5		41.7	40.5	26.8	37.4	39.6	32.1	11.2	17.1	17.1	51.9	20.8
Percentage of M+2	Tricosane	0.0	0.0	0.0	5.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	9.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	5 Me C23	0.0	26.7	5.5	5.1	0.0	6.0	20.5	1.7	2.1	0.0	7.5	0.0	0.0	0.0	6.9	0.0	0.0	2.3	4.5	7.3	0.0	0.0	0.0	18.0	2.5
	Pentacosene	10.0	5.1	4.4	3.7	4.3	3.3	3.6	4.0	3.7	4.6	4.0	2.4	4.5	5.7	4.9	4.0	3.1	0.0	2.6	3.3	8.8	2.7	4.0	3.5	5.5
	11 13 Me C25	18.7	0.0	15.3	10.5	5.3	19.1	9.1	12.6	5.3	0.0	5.9	10.4	10.0	0.0	59.1	12.3	3.6	8.6	16.0	10.1	19.8	5.0	0.0	0.0	12.1
	5 Me C25	5.1	3.6	3.6	1.8	4.4	1.3	1.8	3.8	2.0		0.0	3.3	3.2	1.2	0.0	1.9	2.5	2.3	2.5	1.1	3.9	2.8	0.0	4.8	2.7
	Heptacosene	0.0	0.0	3.4	1.3	0.0	0.0	7.1	5.1	4.9		0.0	0.0	0.0		7.1	0.0	0.0	0.0	0.0	0.0	0.0	9.8	0.0	7.3	0.0

0
0-20
20-40
40-60
60-80
80-100
100+

Figure B.21: Data sheet showing page two of the raw negative control data (propionic acid) for the propionate experiment for *Myrmica sabuleti*.

Propionic Acid (Negative Control)		*Myrmica sabuleti*																						
		51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73
Abundance of M	Tricosane	697	1010	2683	3493	5208	2527	2411	3359	853	1190	1081	3590	635	919	3075	1364	1524	1190	3914	1564	3653	2289	412
	5 Me C23	2003	3357	3141	8343	19648	4399	4241	22760	5468	2777	1559	5922	3199	3980	6473	3566	2274	2843	7942	4714	6214	4881	1777
	Pentacosene	14997	14997	28216	46384	78624	41600	17776	25152	21256	12782	10947	20552	10096	12414	33392	29768	14303	15638	38704	22584	29888	26512	10066
	11 13 Me C25	3236	3236	4738	7814	9822	3695	2694	5967	3362	1742		4625			5530	8262	3016	2462	9355	5431	3934	3209	1393
	5 Me C25	19384	19384	42448	84560	67560	52560	32768	4192	49504	20040	19800	36000	18464	20200	39392	33144	18432	16904	44496	21744	34016	32280	11819
	Heptacosene	532		2055	4468	10186	2215	684		969	719		597	444	1111	3202	1976	926	924	3888	1809	2131	1922	718
Abundance of M+1	Tricosane	246	486	637	971	1907		975	1648	598	365	330	271	189	397	637	407	274	265	890	383	958	577	216
	5 Me C23	520	981	1996	2605	1357	2360	695	6729	1000	237	551	1632	821	1068	968	335	469	596	1963	590	1295	924	403
	Pentacosene	3819	3819	8215	18864	24888	9536	5731	9824	7524	3612	3479	7056	2738	5410	10152	9646	3625	4403	11093	6464	8848	6482	2653
	11 13 Me C25	2192	2192	3156	6438	4519	2993	1313	813	1832	682		1557			3659	4233	1726	1082	5014	3083	1974	1545	585
	5 Me C25	5410	5410	11971	17072	13362	8352	6554	790	8837	3223	3918	7813	3470	4669	9430	6402	4239	3999	8513	4944	7500	8417	2914
	Heptacosene			214	2461	2291	1154	463		399	480		179	178	156	1041	420	298	262	1505	560	818	531	
Abundance of M+2	Tricosane																							
	5 Me C23	255			177			154	377		187						294			164		192		
	Pentacosene	1056	1056	847	4265	2441	1494	190	363	428	433	492	537	251	666	1458	1640	592	707	1026	639	1334	881	273
	11 13 Me C25	782	782	565	5605	728		227	165	208						615	164	208	204	671	287	376	180	
	5 Me C25	525	525	2071	809	3271	1911	890		1417	599	230	799	550	516	1313	613	435	361	1103	491	878	851	473
	Heptacosene					261																		
Percentage of M+1	Tricosane	35.3	48.1	23.7	27.8	36.6	0.0	40.4	49.1	70.1	30.7	30.5	7.5	29.8	43.2	20.7	29.8	18.0	22.3	22.7	24.5	26.2	25.2	52.4
	5 Me C23	26.0	29.2	63.5	31.2	6.9	53.6	16.4	29.6	18.3	8.5	35.3	27.6	25.7	26.8	15.0	9.4	20.6	21.0	24.7	12.5	20.8	18.9	22.7
	Pentacosene	25.5	25.5	29.1	40.7	31.7	22.9	32.2	39.1	35.4	28.3	31.8	34.3	27.1	43.6	30.4	32.4	25.3	28.2	28.7	28.6	29.6	24.4	26.4
	11 13 Me C25	67.7	67.7	66.6	82.4	46.0	81.0	48.7	13.6	54.5	39.2		33.7			66.2	51.2	57.2	43.9	53.6	56.8	50.2	48.1	42.0
	5 Me C25	27.9	27.9	28.2	20.2	19.8	15.9	20.0	18.8	17.9	16.1	19.8	21.7	18.8	23.1	23.9	19.3	23.0	23.7	19.1	22.7	22.0	26.1	24.7
	Heptacosene	0.0		10.4	55.1	22.5	52.1	67.7		41.2	66.8		30.0	40.1	14.0	32.5	21.3	32.2	28.4	38.7	31.0	38.4	27.6	0.0
Percentage of M+2	Tricosane	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	5 Me C23	12.7	0.0	0.0	2.1	0.0	0.0	3.6	1.7	0.0	6.7	0.0	0.0	0.0	0.0	0.0	8.2	0.0	0.0	2.1	0.0	3.1	0.0	0.0
	Pentacosene	7.0	7.0	3.0	9.2	3.1	3.6	1.1	1.4	2.0	3.4	4.5	2.6	2.5	5.4	4.4	5.5	4.1	4.5	2.7	2.8	4.5	3.3	2.7
	11 13 Me C25	24.2	24.2	11.9	71.7	7.4	0.0	8.4	2.8	6.2	0.0		0.0			11.1	2.0	6.9	8.3	7.2	5.3	9.6	5.6	0.0
	5 Me C25	2.7	2.7	4.9	1.0	4.8	3.6	2.7	0.0	2.9	3.0	1.2	2.2	3.0	2.6	3.3	1.8	2.4	2.1	2.5	2.3	2.6	2.6	4.0
	Heptacosene	0.0		0.0	0.0	2.6	0.0	0.0		0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

0
0-20
20-40
40-60
60-80
80-100
100+

Figure B.22: Data sheet showing page three of the raw negative control data (propionic acid) for the propionate experiment for *Myrmica sabuleti*.

Sodium [1-^{13}C]acetate (Positive Control)		*Myrmica sabuleti*																								
		Con 1	Con 2	Con 3	Con 4	Con 5	Con 6	Con 7	Con 8	Con 9	Con 10	Con 11	Con 12	Con 13	Con 14	Con 15	Con 16	Con 17	Con 18	Con 19	Con 20	Con 21	Con 22	Con 23	Con 24	Con 25
Abundance of M	Tricosane	2741	1530	446	1061	1507	581	935	307	177	167	686	416	866	842	1341	864	1272	408	2791	616	813	546	1781	716	978
	5 Me C23	5887	4686	836	2912	3986	1352	2068	839	930		1841	1230	1607	1822	4088	2293	3629	1268	5628	1778	2054	1075	3040	1924	2756
	Pentacosene	36464	25032	5633	19896	20464	12328	14430	5944	5770	3709	11220	8266	12386	11758	23464	14535	19584	8249	27960	10972	14013	9604	25440	12658	16408
	11 13 Me C25	11223	7143	2344	4231	8334	5694	4286	1627	1547	1141	5472	2554	4067	4114	12725	6598	7405	3290	10259	3480	6853	3687	5918	4735	6492
	5 Me C25	33192	28304	5146	17456	23208	12340	17000	5591	7762	4080	10698	8675	14303	10761	21136	12390	21104	6140	37944	11305	12949	7920	28240	12970	15138
	Heptacosene	5518	3763	739	2382	2859	1437	2178	409	599	420	1607	1085	1778	1482	4411	1698	2593	745	4857	1337	2335	1192	3406	1817	2545
Abundance of M+1	Tricosane	1786	1158	252	629	1046	208	632		271		346	226	680	451	770	417	897		1353	488	451	273	1425	513	682
	5 Me C23	1931	2021	463	1157	1659	328	823	381	629		639	464	780	643	1158	694	1299	343	2924	947	639	302	1903	720	955
	Pentacosene	18168	12445	2800	9440	9774	4992	7926	3185	4366	2391	5114	4188	7178	4732	9610	6197	9751	3986	16512	7413	5727	4593	14951	6005	7528
	11 13 Me C25	6555	5197	1435	3591	5499	4149	2614	1093	1575	997	3845	1632	2812	2760	8074	4317	5673	2369	7680	2858	4698	2266	4465	2986	4316
	5 Me C25	15429	14315	2230	7507	11410	3364	8392	3205	5092	2004	4433	3890	6957	3686	7690	4797	8812	2620	20232	7840	4521	3178	16298	5297	5643
	Heptacosene	3260	2228	442	1553	1855	816	1417	380	530	303	761	581	1258	825	1689	1089	1374	556	3392	886	743	666	1971	629	1340
Abundance of M+2	Tricosane	808	660	239	339	479		383		188			188	262	152	357	246	502		1213	263		264	543	191	206
	5 Me C23	788	965	164	364	671		257	330	400		269		397	207	309		638		1174	419	151	162	575	282	297
	Pentacosene	7436	7244	1591	3981	4652	1675	3973	1927	2662	1396	2081	2236	4036	2025	2795	2436	3823	1308	7901	3253	2194	1927	8237	2124	3366
	11 13 Me C25	2021	1769	675	1418	1945	1092	1027	451	535	316	1162	413	967	867	2009	1349	1529	796	3015	1125	964	592	1507	1135	1531
	5 Me C25	6188	8297	992	3060	4863	1193	4030	1170	3326	1382	1276	1975	3085	1320	2473	1484	3623	921	10515	3789	1881	1448	8369	2347	2681
	Heptacosene	1492	963	233	293	856	200	504	192	243	161	315	277	619	271	824	613	636	241	1819	479	438	332	1171	350	403
Percentage of M+1	Tricosane	65.2	75.7	56.5	59.3	69.4	35.8	67.6	0.0	153.1	0.0	50.4	54.3	78.5	53.6	57.4	48.3	70.5	0.0	48.5	79.2	55.5	50.0	80.0	71.6	69.7
	5 Me C23	32.8	43.1	55.4	39.7	41.6	24.3	39.8	45.4	67.6		34.7	37.7	48.5	35.3	28.3	30.3	35.8	27.1	52.0	53.3	31.1	28.1	62.6	37.4	34.7
	Pentacosene	49.8	49.7	49.7	47.4	47.8	40.5	54.9	53.6	75.7	64.5	45.6	50.7	58.0	40.2	41.0	42.6	49.8	48.3	59.1	67.6	40.9	47.8	58.8	47.4	45.9
	11 13 Me C25	58.4	72.8	61.2	84.9	66.0	72.9	61.0	67.2	101.8	87.4	70.3	63.9	69.1	67.1	63.4	65.4	76.6	72.0	74.9	82.1	68.6	61.5	75.4	63.1	66.5
	5 Me C25	46.5	50.6	43.3	43.0	49.2	27.3	49.4	57.3	65.6	49.1	41.4	44.8	48.6	34.3	36.4	38.7	41.8	42.7	53.3	69.3	34.9	40.1	57.7	40.8	37.3
	Heptacosene	59.1	59.2	59.8	65.2	64.9	56.8	65.1	92.9	88.5	72.1	47.4	53.5	70.8	55.7	38.3	64.1	53.0	74.6	69.8	66.3	31.8	55.9	57.9	34.6	52.7
Percentage of M+2	Tricosane	29.5	43.1	53.6	32.0	31.8	0.0	41.0	0.0	106.2	0.0	0.0	45.2	30.3	18.1	26.6	28.5	39.5	0.0	43.5	42.7	0.0	48.4	30.5	26.7	21.1
	5 Me C23	13.4	20.6	19.6	12.5	16.8	0.0	12.4	39.3	43.0		14.6	0.0	24.7	11.4	7.6	0.0	17.6	0.0	20.9	23.6	7.4	15.1	18.9	14.7	10.8
	Pentacosene	20.4	28.9	28.2	20.0	22.7	13.6	27.5	32.4	46.1	37.6	18.5	27.1	32.6	17.2	11.9	16.8	19.5	15.9	28.3	29.6	15.7	20.1	32.4	16.8	20.5
	11 13 Me C25	18.0	24.8	28.8	33.5	23.3	19.2	24.0	27.7	34.6	27.7	21.2	16.2	23.8	21.1	15.8	20.4	20.6	24.2	29.4	32.3	14.1	16.1	25.5	24.0	23.6
	5 Me C25	18.6	29.3	19.3	17.5	21.0	9.7	23.7	20.9	42.8	33.9	11.9	22.8	21.6	12.3	11.7	12.0	17.2	15.0	27.7	33.5	14.5	18.3	29.6	18.1	17.7
	Heptacosene	27.0	25.6	31.5	12.3	29.9	13.9	23.1	46.9	40.6	38.3	19.6	25.5	34.8	18.3	18.7	36.1	24.5	32.3	37.5	35.8	18.8	27.9	34.4	19.3	15.8

0
0-20
20-40
40-60
60-80
80-100
100+

Figure B.23: Data sheet showing page one of the raw positive control data (sodium [1-^{13}C]acetate) for the propionate experiment for *Myrmica sabuleti*.

Sodium [1-^{13}C]acetate (Positive Control)		*Myrmica sabuleti*																	
		Con 26	Con 27	Con 28	Con 29	Con 30	Con 31	Con 32	Con 33	Con 34	Con 35	Con 36	Con 37	Con 38	Con 39	Con 40	Con 41	Con 42
Abundance of M	Tricosane	2424	1391	586	902	1085	2395	1552	2037	4966	2712	3229	5273	1696	4535	2778	2442	1370
	5 Me C23	6249	2727	1078	1718	3996	4596	3013	11013	7944	2458	4663	9988	9988	14346	10659	5544	5389
	Pentacosene	42608	18696	12026	17696	28336	27312	22448	70184	56776	37528	54736	61120	61120	64280	35000	33376	42232
	11 13 Me C25	19968	5385	6255	4228	10541	14707	9518	14919	24272	8397	14393	3960	23928	19544	12717	12457	8096
	5 Me C25	39168	19144	7425	21944	27344	23928	18480	47528	62640	28640	30296	11081	63432	44536	45432	30936	34928
	Heptacosene	7008	2920	664	1774	5420	6843	3189	5197	7293	4824	10928	2270	11208	10845	7209	5786	2703
Abundance of M+1	Tricosane	1142	959	344	414	1199	1445	1369	502	2887	223	932	2063	2188	3073	1714	689	340
	5 Me C23	1579	964	348	391	1328	2003	962	2828	2535	431	1337	2746	2746	4083	2398	1393	575
	Pentacosene	14489	9904	4491	8919	10391	12297	8843	24040	29128	16776	20208	26408	26408	18504	18200	22176	24760
	11 13 Me C25	12859	3637	2877	2568	6571	12298	2906	17544	12213	3322	6814	2933	16271	13377	7989	6265	5158
	5 Me C25	9687	9902	3656	8380	9226	11191	6216	17656	24944	14708	13492	6488	28720	14676	14130	14187	20320
	Heptacosene	2927	1514	1219	1594	1796	2238	2477	3887	5446	1955	3055	1052	5648	4698	1442	2896	3274
Abundance of M+2	Tricosane	331	661			226	267			1303	176		1181	1491	1234	498		375
	5 Me C23	329	564			553	653	559		820		569		3862	821	602	772	
	Pentacosene	3607	4153	846	2849	3146	4457	2975	6445	13746	3944	8874	15208	15208	8675	4276	7109	6054
	11 13 Me C25	2767	1392	477	1221	3460	3293	1134	4366	2540	1671	4182	552	3915	5047	1915	2584	918
	5 Me C25	2753	5932	2334	5680	4693	3752	2570	6686	6952	9405	5539	2590	13016	7071	6173	3955	7428
	Heptacosene	923	717		1097	661	846	1401	1354	2193	812	1396	632	2081	1593	527	421	2079
Percentage of M+1	Tricosane	47.1	68.9	58.7	45.9	110.5	60.3	88.2	24.6	58.1	8.2	28.9	39.1	129.0	67.8	61.7	28.2	24.8
	5 Me C23	25.3	35.4	32.3	22.8	33.2	43.6	31.9	25.7	31.9	17.5	28.7	27.5	27.5	28.5	22.5	25.1	10.7
	Pentacosene	34.0	53.0	37.3	50.4	36.7	45.0	39.4	34.3	51.3	44.7	36.9	43.2	43.2	28.8	52.0	66.4	58.6
	11 13 Me C25	64.4	67.5	46.0	60.7	62.3	83.6	30.5	117.6	50.3	39.6	47.3	74.1	68.0	68.4	62.8	50.3	63.7
	5 Me C25	24.7	51.7	49.2	38.2	33.7	46.8	33.6	37.1	39.8	51.4	44.5	58.6	45.3	33.0	31.1	45.9	58.2
	Heptacosene	41.8	51.8	183.6	89.9	33.1	32.7	77.7	74.8	74.7	40.5	28.0	46.3	50.4	43.3	20.0	50.1	121.1
Percentage of M+2	Tricosane	13.7	47.5	0.0	0.0	20.8	11.1	0.0	0.0	26.2	6.5	0.0	22.4	87.9	27.2	17.9	0.0	27.4
	5 Me C23	5.3	20.7	0.0	0.0	13.8	14.2	18.6	0.0	10.3	0.0	12.2	0.0	38.7	5.7	5.6	13.9	0.0
	Pentacosene	8.5	22.2	7.0	16.1	11.1	16.3	13.3	9.2	24.2	10.5	16.2	24.9	24.9	13.5	12.2	21.3	14.3
	11 13 Me C25	13.9	25.8	7.6	28.9	32.8	22.4	11.9	29.3	10.5	19.9	29.1	13.9	16.4	25.8	15.1	20.7	11.3
	5 Me C25	7.0	31.0	31.4	25.9	17.2	15.7	13.9	14.1	11.1	32.8	18.3	23.4	20.5	15.9	13.6	12.8	21.3
	Heptacosene	13.2	24.6	0.0	61.8	12.2	12.4	43.9	26.1	30.1	16.8	12.8	27.8	18.6	14.7	7.3	7.3	76.9

0
0-20
20-40
40-60
60-80
80-100
100+

Figure B.24: Data sheet showing page two of the raw positive control data (sodium [1-^{13}C]acetate) for the propionate experiment for *Myrmica sabuleti*.

Propionic-2,2-d_2 acid-d (Substrate)		*Myrmica sabuleti*																								
		Sub 1	Sub 2	Sub 3	Sub 4	Sub 5	Sub 6	Sub 7	Sub 8	Sub 9	Sub 10	Sub 11	Sub 12	Sub 13	Sub 14	Sub 15	Sub 16	Sub 17	Sub 18	Sub 19	Sub 20	Sub 21	Sub 22	Sub 23	Sub 24	Sub 25
Abundance of M	Tricosane	2113	3486	2913	1896	1468	2206	7441	2681	3543	3044	3898	1988	11725	7814	7325	5578	5327	2963	2616	3582	3570	2495	2777	1917	909
	5 Me C23	1514	7283	6603	3673	1246	3549	13021	6084	4107	5924	10352	3861	18784	12024	17960	8075	11691	5466	3498	4822	6053	4215	4205		796
	Pentacosene	10184	24840	24344	16129	9232	16243	38840	22096	15121	21864	60064	56248	74192	27080	57304	33200	40288	27392	19392	24824	26144	19408	57712	40424	16011
	11 13 Me C25	2970	11800	10754	7478	736	694	2843	12406	1524	1556	21760	9331	13921	18392	19824	18848	18592	10629	7402	12444	11575	8227	14384	6037	4600
	5 Me C25	13829	32144	29400	23176	11318	6709	49464	29536	21824	29328	89592	56616	41144	28304	42992	52624	50880	32264	20928	27456	31184	23920	55816	26752	14917
	Heptacosene	1829	5089	4967	3293	1206	3281	8574	4665	2586	4362	8828	4021	11320	8343	8884	10100	9425	5948	3842	5525	5317	4274	6257	4131	1664
Abundance of M+1	Tricosane	682	727	867	560	280	586	1812	744	1166	747	803	1122	3158	1828	1886	1452	1714	716	639	1044	1343	748	1209	296	404
	5 Me C23	286	1389	1469	900	265	802	3031	1427	891	1361	1914	1403	4418	2523	3972	1605	2509	1103	796	1198	1409	1023	2404		383
	Pentacosene	2756	6710	6425	4136	2730	4025	11983	6323	4822	5945	19528	18136	21960	7030	16126	8916	11697	7517	5077	6787	6938	5182	14059	11286	5097
	11 13 Me C25	1766	7269	7138	4608	201	284	855	7148	398	474	10690	5890	7807	9637	10967	10279	9775	6499	4495	7454	7109	3882	7965	4909	2299
	5 Me C25	3214	7322	6685	5219	2768	3733	11484	6667	5089	6321	14912	16520	9096	5724	9516	11784	11577	7715	4490	6470	6962	5168	13465	5378	2241
	Heptacosene	547	1657	1353	1095	438	1031	3300	1781	855	1525	2763	2057	3830	2175	2649	3583	2762	1658	1267	1893	1896	1137	3220	1917	176
Abundance of M+2	Tricosane					160		275		166				381	330	301		173				165		276		213
	5 Me C23		223		154	501		386	215					440	274	522	186	376	173							
	Pentacosene	460	1034	874	675		538	1477	928	680	838	2918	3427	3443	754	2394	1566	1736	1050	621	1084	968	910	4334	1507	914
	11 13 Me C25	211	863	786	555				1159			4800	556	802	1437	1307	1551	1169	843	629	915	1139	704	1689	2780	
	5 Me C25	426	813	827	581	413	470	1428	762	491	624	2710	1065	730	658	981	1548	1551	720	538	770	911	504	1468	433	339
	Heptacosene		214	219	160			306	294	240	254	262		466	257	305	536	511	326	284	276		292			
Percentage of M+1	Tricosane	32.3	20.9	29.8	29.5	19.1	26.6	24.4	27.8	32.9	24.5	20.6	56.4	26.9	23.4	25.7	26.0	32.2	24.2	24.4	29.1	37.6	30.0	43.5	15.4	44.4
	5 Me C23	18.9	19.1	22.2	24.5	21.3	22.6	23.3	23.5	21.7	23.0	18.5	36.3	23.5	21.0	22.1	19.9	21.5	20.2	22.8	24.8	23.3	24.3	57.2		48.1
	Pentacosene	27.1	27.0	26.4	25.6	29.6	24.8	30.9	28.6	31.9	27.2	32.5	32.2	29.6	26.0	28.1	26.9	29.0	27.4	26.2	27.3	26.5	26.7	24.4	27.9	31.8
	11 13 Me C25	59.5	61.6	66.4	61.6	27.3	40.9	30.1	57.6	26.1	30.5	49.1	63.1	56.1	52.4	55.3	54.5	52.6	61.1	60.7	59.9	61.4	47.2	55.4	81.3	50.0
	5 Me C25	23.2	22.8	22.7	22.5	24.5	55.6	23.2	22.6	23.3	21.6	16.6	29.2	22.1	20.2	22.1	22.4	22.8	23.9	21.5	23.6	22.3	21.6	24.1	20.1	15.0
	Heptacosene	29.9	32.6	27.2	33.3	36.3	31.4	38.5	38.2	33.1	35.0	31.3	51.2	33.8	26.1	29.8	35.5	29.3	27.9	33.0	34.3	35.7	26.6	51.5	46.4	10.6
Percentage of M+2	Tricosane	0.0	0.0	0.0	0.0	10.9	0.0	3.7	0.0	4.7	0.0	0.0	0.0	3.2	4.2	4.1	0.0	3.2	0.0	0.0	0.0	4.6	0.0	9.9	0.0	23.4
	5 Me C23	0.0	3.1	0.0	4.2	40.2	0.0	3.0	3.5	0.0	0.0	0.0	0.0	2.3	2.3	2.9	2.3	3.2	3.2	0.0	0.0	0.0	0.0	0.0		0.0
	Pentacosene	4.5	4.2	3.6	4.2	0.0	3.3	3.8	4.2	4.5	3.8	4.9	6.1	4.6	2.8	4.2	4.7	4.3	3.8	3.2	4.4	3.7	4.7	7.5	3.7	5.7
	11 13 Me C25	7.1	7.3	7.3	7.4	0.0	0.0	0.0	9.3	0.0	0.0	22.1	6.0	5.8	7.8	6.6	8.2	6.3	7.9	8.5	7.4	9.8	8.6	11.7	46.0	0.0
	5 Me C25	3.1	2.5	2.8	2.5	3.6	7.0	2.9	2.6	2.2	2.1	3.0	1.9	1.8	2.3	2.3	2.9	3.0	2.2	2.6	2.8	2.9	2.1	2.6	1.6	2.3
	Heptacosene	0.0	4.2	4.4	4.9	0.0	0.0	3.6	6.3	9.3	5.8	3.0	0.0	4.1	3.1	3.4	5.3	5.4	5.5	7.4	5.0	0.0	6.8	0.0	0.0	0.0

0
0-20
20-40
40-60
60-80
80-100
100+

Figure B.25: Data sheet showing page one of the raw substrate data (propionic-2,2-d_2 acid-d) for the propionate experiment for *Myrmica sabuleti*.

Propionic-2,2-d_2 acid-d (Substrate)		*Myrmica sabuleti*																								
		Sub 26	Sub 27	Sub 28	Sub 29	Sub 30	Sub 31	Sub 32	Sub 33	Sub 34	Sub 35	Sub 36	Sub 37	Sub 38	Sub 39	Sub 40	Sub 41	Sub 42	Sub 43	Sub 44	Sub 45	Sub 46	Sub 47	Sub 48	Sub 49	Sub 50
Abundance of M	Tricosane	1014	3673	2735	2339	1883	2397	1877	2518	2532	2656	5562	1128	992	2776	1240	4538	3285	10333	5162	8765	11123	4212	6582	4685	10559
	5 Me C23	1475	1794	2676	2784	1246	3172	1842	3713	4163	4493	10577	2083	1458	4939	4078	8912	5867	23696	8890	15056	28824	11383	9363	11453	19608
	Pentacosene	22440	10531	14463	12163	7423	15275	10478	17432	18800	18656	38992	28792	15428	54640	41160	42168	27816	76208	41008	60760	71160	45656	43624	48848	72632
	11 13 Me C25	3164	2430	4611	4485	1811	7303	3470	7841	7923	6330	16416	6963	3606	6725	5991	16416	13868	36944	20096	20504	41768	19448	17192	18856	35368
	5 Me C25	20184	12842	18120	15664	10515	21696	11006	22360	25416	24872	47688	28760	12821	48440	23152	54424	29368	96448	48496	71368	106776	55504	47320	64288	83432
	Heptacosene	1067	1595	3079	2389	1042	3346	1713	3907	3728	3780	9014	1826	355	3519	2557	7677	4384	16584	7818	11024	18200	5759	5047	5171	14047
Abundance of M+1	Tricosane		931	555	795	533	549	382	614	624	816	1601	365	153	1143	935	1170	838	2463	1223	2684	2827	1300	1878	1370	2644
	5 Me C23	959	447	566	620	338	750	404	833	1017	1248	2499		622	670	1230	2102	1065	4665	1687	3717	5956	2392	1862	2270	4324
	Pentacosene	4825	3096	3946	3364	2154	4310	2984	5357	5236	5196	10206	7732	6832	13731	13694	11318	7587	22000	11249	16536	21520	12282	12297	14261	22032
	11 13 Me C25	2032	1480	2551	2756	932	4143	1767	4340	4816	3616	9059	3530	687	5491	3482	8759	7556	19344	11143	12063	23240	10771	9688	10184	18496
	5 Me C25	3836	2952	4344	3426	2179	4545	2692	4926	5008	5806	10528	7478	5678	10179	10179	12511	7606	21712	10997	16193	23848	11917	11334	14810	19592
	Heptacosene	534	540	1111	539	443	1043	650	1473	1251	1187	2553	1232	280	1834	920	2342	1548	5234	2502	3493	5396	1658	1877	1678	5015
Abundance of M+2	Tricosane											154						184	336	162	393	478		194	232	351
	5 Me C23						159				155	240					269		428	152	403	656	324	170	256	431
	Pentacosene	1677	451	617	299	381	409	386	817	712	579	1190	1148	1158	1606	1131	1319	973	3006	1510	2015	3323	1554	1637	1928	2614
	11 13 Me C25	900	241	331	340	150	568	259	643	682	424	1217	1245		434	963	1146	1046	2907	1759	1378	2899	1161	1198	1400	2711
	5 Me C25		425	538	435	314	718	167	674	393	830	1008	1730	206	1781	1319	1400	768	1843	1674	1980	2774	1331	1388	1558	1931
	Heptacosene	282		218	158			162	160		208	459	439		646		368	180	851	293	542	987	291	265	283	430
Percentage of M+1	Tricosane	0.0	25.3	20.3	34.0	28.3	22.9	20.4	24.4	24.6	30.7	28.8	32.4	15.4	41.2	75.4	25.8	25.5	23.8	23.7	30.6	25.4	30.9	28.5	29.2	25.0
	5 Me C23	65.0	24.9	21.2	22.3	27.1	23.6	21.9	22.4	24.4	27.8	23.6	0.0	42.7	13.6	30.2	23.6	18.2	19.7	19.0	24.7	20.7	21.0	19.9	19.8	22.1
	Pentacosene	21.5	29.4	27.3	27.7	29.0	28.2	28.5	30.7	27.9	27.9	26.2	26.9	44.3	25.1	33.3	26.8	27.3	28.9	27.4	27.2	30.2	26.9	28.2	29.2	30.3
	11 13 Me C25	64.2	60.9	55.3	61.4	51.5	56.7	50.9	55.4	60.8	57.1	55.2	50.7	19.1	81.7	58.1	53.4	54.5	52.4	55.4	58.8	55.6	55.4	56.4	54.0	52.3
	5 Me C25	19.0	23.0	24.0	21.9	20.7	20.9	24.5	22.0	19.7	23.3	22.1	26.0	44.3	21.0	44.0	23.0	25.9	22.5	22.7	22.7	22.3	21.5	24.0	23.0	23.5
	Heptacosene	50.0	33.9	36.1	22.6	42.5	31.2	37.9	37.7	33.6	31.4	28.3	67.5	78.9	52.1	36.0	30.5	35.3	31.6	32.0	31.7	29.6	28.8	37.2	32.5	35.7
Percentage of M+2	Tricosane	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.8	0.0	0.0	0.0	0.0	0.0	5.6	3.3	3.1	4.5	4.3	0.0	2.9	5.0	3.3
	5 Me C23	0.0	0.0	0.0	0.0	0.0	5.0	0.0	0.0	0.0	3.4	2.3	0.0	0.0	0.0	0.0	3.0	0.0	1.8	1.7	2.7	2.3	2.8	1.8	2.2	2.2
	Pentacosene	7.5	4.3	4.3	2.5	5.1	2.7	3.7	4.7	3.8	3.1	3.1	4.0	7.5	2.9	2.7	3.1	3.5	3.9	3.7	3.3	4.7	3.4	3.8	3.9	3.6
	11 13 Me C25	28.4	9.9	7.2	7.6	8.3	7.8	7.5	8.2	8.6	6.7	7.4	17.9	0.0	6.5	16.1	7.0	7.5	7.9	8.8	6.7	6.9	6.0	7.0	7.4	7.7
	5 Me C25	0.0	3.3	3.0	2.8	3.0	3.3	1.5	3.0	1.5	3.3	2.1	6.0	1.6	3.7	5.7	2.6	2.6	1.9	3.5	2.8	2.6	2.4	2.9	2.4	2.3
	Heptacosene	26.4	0.0	7.1	6.6	0.0	0.0	9.5	4.1	0.0	5.5	5.1	24.0	0.0	18.4	0.0	4.8	4.1	5.1	3.7	4.9	5.4	5.1	5.3	5.5	3.1

0
0-20
20-40
40-60
60-80
80-100
100+

Figure B.26: Data sheet showing page two of the raw substrate data (propionic-2,2-d_2 acid-d) for the propionate experiment for *Myrmica sabuleti*.

Propionic-2,2-d_2 acid-d (Substrate)		*Myrmica sabuleti*																								
		Sub 51	Sub 52	Sub 53	Sub 54	Sub 55	Sub 56	Sub 57	Sub 58	Sub 59	Sub 60	Sub 61	Sub 62	Sub 63	Sub 64	Sub 65	Sub 66	Sub 67	Sub 68	Sub 69	Sub 70	Sub 71	Sub 72	Sub 73	Sub 74	Sub 75
Abundance of M	Tricosane	4468	1654	3219	4649	3415	2586	3175	2818	5436	3228	2501	1467	2887	2014	7564	3108	6747	5367	7327	13373	2667	8110	2029	1543	2506
	5 Me C23	8595	1360	6147	6781	5257	3435	4676	4395	10513	3333	5020	3372	7801	4522	9649	3344	11133	10602	14276	32888	5932	15743	3017	1070	4683
	Pentacosene	40624	17880	35275	53992	33360	27072	36080	33600	52536	35484	32696	17544	31744	24168	65992	24568	57704	50984	59824	108376	23800	71352	15326	8195	31328
	11 13 Me C25	13769	5251	10692	17112	9847	4568	9589	12402	20320	6353	9883	8085	13286	6610	16648	7871	24800	17224	23208	46280	11948	31920	4212	1927	9063
	5 Me C25	44648	14850	39784	65920	39208	26496	43384	34240	55800	37968	31176	19120	40136	26904	79400	24248	61696	64264	77720	134400	28888	89184	23256	11550	35216
	Heptacosene	5033	1432	5365	8337	4710	2628	4783	4130	7507	4094	3208	2328	4853	3656	10914	2234	9525	9503	10979	24752	3362	14150	1944	847	4404
Abundance of M+1	Tricosane	1077	571	994	1268	901	617	897	1089	1498	749	706	423	889	578	2210	1019	2135	1336	1743	4027	728	2429	530	412	729
	5 Me C23	1683	275	1189	1689	1030	672	1113	1040	3002	981	1139	739	1889	968	2017	780	2615	2357	3279	6228	1096	3812	559	191	896
	Pentacosene	10497	4863	10903	14102	10136	8534	10025	10223	14078	10289	8783	5422	9245	6622	19352	6919	15015	14637	16800	28560	7521	19528	4215	2577	8881
	11 13 Me C25	7572	3009	5202	9856	5826	2740	5635	5960	9276	3990	4681	3967	6651	3988	8868	4621	14011	8550	12609	25912	6654	15594	2756	1016	4882
	5 Me C25	8850	3563	9579	13939	8243	5321	10389	6773	13782	9088	7974	4220	9240	6023	18680	5842	14949	15486	16384	30232	6428	19328	5830	2658	8682
	Heptacosene	1754	397	1915	3100	1583	713	1682	1128	2766	1122	1186	782	1908	1185	3814	964	3159	3124	3815	7546	1248	4368	724	209	1465
Abundance of M+2	Tricosane	270			382	182				271			159			284		271	272	217	427	207	343			199
	5 Me C23	188		157	229					329				211		174		387	335	409	946	163	368			195
	Pentacosene	1483	571	1275	2077	1687	1241	1605	1393	1973	1504	1247	689	1017	851	3342	1047	2110	2248	2248	4384	1108	2724	543	347	1102
	11 13 Me C25	948	413	606	1595	694	262	674	1040	1444	421	919	492	1011	669	1599	700	1660	1143	1549	3417	923	2421	334	198	957
	5 Me C25	1161	397	1295	1800	998	984	1573	960	1068	1112	1034	453	989	628	2686	480	1570	1848	1805	3454	850	2236	531	350	991
	Heptacosene	274		255	467	273	203	248	232	458	247	253		269	176	472		351	351	672	1153	262	849			302
Percentage of M+1	Tricosane	24.1	34.5	30.9	27.3	26.4	23.9	28.3	38.6	27.6	23.2	28.2	28.8	30.8	28.7	29.2	32.8	31.6	24.9	23.8	30.1	27.3	30.0	26.1	26.7	29.1
	5 Me C23	19.6	20.2	19.3	24.9	19.6	19.6	23.8	23.7	28.6	29.4	22.7	21.9	24.2	21.4	20.9	23.3	23.5	22.2	23.0	18.9	18.5	24.2	18.5	17.9	19.1
	Pentacosene	25.8	27.2	30.9	26.1	30.4	31.5	27.8	30.4	26.8	29.0	26.9	30.9	29.1	27.4	29.3	28.2	26.0	28.7	28.1	26.4	31.6	27.4	27.5	31.4	28.3
	11 13 Me C25	55.0	57.3	48.7	57.6	59.2	60.0	58.8	48.1	45.6	62.8	47.4	49.1	50.1	60.3	53.3	58.7	56.5	49.6	54.3	56.0	55.7	48.9	65.4	52.7	53.9
	5 Me C25	19.8	24.0	24.1	21.1	21.0	20.1	23.9	19.8	24.7	23.9	25.6	22.1	23.0	22.4	23.5	24.1	24.2	24.1	21.1	22.5	22.3	21.7	25.1	23.0	24.7
	Heptacosene	34.8	27.7	35.7	37.2	33.6	27.1	35.2	27.3	36.8	27.4	37.0	33.6	39.3	32.4	34.9	43.2	33.2	32.9	34.7	30.5	37.1	30.9	37.2	24.7	33.3
Percentage of M+2	Tricosane	6.0	0.0	0.0	8.2	5.3	0.0	0.0	0.0	5.0	0.0	0.0	10.8	0.0	0.0	3.8	0.0	4.0	5.1	3.0	3.2	7.8	4.2	0.0	0.0	7.9
	5 Me C23	2.2	0.0	2.6	3.4	0.0	0.0	0.0	0.0	3.1	0.0	0.0	0.0	2.7	0.0	1.8	0.0	3.5	3.2	2.9	2.9	2.7	2.3	0.0	0.0	4.2
	Pentacosene	3.7	3.2	3.6	3.8	5.1	4.6	4.4	4.1	3.8	4.2	3.8	3.9	3.2	3.5	5.1	4.3	3.7	4.4	3.8	4.0	4.7	3.8	3.5	4.2	3.5
	11 13 Me C25	6.9	7.9	5.7	9.3	7.0	5.7	7.0	8.4	7.1	6.6	9.3	6.1	7.6	10.1	9.6	8.9	6.7	6.6	6.7	7.4	7.7	7.6	7.9	10.3	10.6
	5 Me C25	2.6	2.7	3.3	2.7	2.5	3.7	3.6	2.8	1.9	2.9	3.3	2.4	2.5	2.3	3.4	2.0	2.5	2.9	2.3	2.6	2.9	2.5	2.3	3.0	2.8
	Heptacosene	5.4	0.0	4.8	5.6	5.8	7.7	5.2	5.6	6.1	6.0	7.9	0.0	5.5	4.8	4.3	0.0	3.7	3.7	6.1	4.7	7.8	6.0	0.0	0.0	6.9

0
0-20
20-40
40-60
60-80
80-100
100+

Figure B.27: Data sheet showing page three of the raw substrate data (propionic-2,2-d_2 acid-d) for the propionate experiment for *Myrmica sabuleti*.

Propionic-2,2-d_2 acid-d (Substrate)		*Myrmica sabuleti*				
		Sub 76	Sub 77	Sub 78	Sub 79	Sub 80
Abundance of M	Tricosane	6458	1802	4061	2550	2939
	5 Me C23	13416	3462	5778	2772	4576
	Pentacosene	87424	42608	61296	29752	50112
	11 13 Me C25	15709	11476	12585	4781	12513
	5 Me C25	110336	28920	49000	19376	38736
	Heptacosene	7681	4714	9122	4129	7408
Abundance of M+1	Tricosane	2191	527	1711	484	1653
	5 Me C23	1803	765	2035	473	1586
	Pentacosene	29144	13966	12157	10930	12217
	11 13 Me C25	10253	6484	11492	3170	8876
	5 Me C25	21680	9644	7049	6066	8014
	Heptacosene	2974	3080	2961	902	2629
Abundance of M+2	Tricosane	203				
	5 Me C23	2212	692			586
	Pentacosene	2877	587	2556	1172	3748
	11 13 Me C25	1189	1600	1149	1811	1327
	5 Me C25	2399	806	1112	408	515
	Heptacosene	655		242		
Percentage of M+1	Tricosane	33.9	29.2	42.1	19.0	56.2
	5 Me C23	13.4	22.1	35.2	17.1	34.7
	Pentacosene	33.3	32.8	19.8	36.7	24.4
	11 13 Me C25	65.3	56.5	91.3	66.3	70.9
	5 Me C25	19.6	33.3	14.4	31.3	20.7
	Heptacosene	38.7	65.3	32.5	21.8	35.5
Percentage of M+2	Tricosane	3.1	0.0	0.0	0.0	0.0
	5 Me C23	16.5	20.0	0.0	0.0	12.8
	Pentacosene	3.3	1.4	4.2	3.9	7.5
	11 13 Me C25	7.6	13.9	9.1	37.9	10.6
	5 Me C25	2.2	2.8	2.3	2.1	1.3
	Heptacosene	8.5	0.0	2.7	0.0	0.0

0
0-20
20-40
40-60
60-80
80-100
100+

Figure B.28: Data sheet showing page four of the raw substrate data (propionic-2,2-d_2 acid-d) for the propionate experiment for *Myrmica sabuleti*.

Propionic Acid (Negative Control)		*Myrmica scabrinodis*																								
		Con 1	Con 2	Con 3	Con 4	Con 5	Con 6	Con 7	Con 8	Con 9	Con 10	Con 11	Con 12	Con 13	Con 14	Con 15	Con 16	Con 17	Con 18	Con 19	Con 20	Con 21	Con 22	Con 23	Con 24	Con 25
Abundance of M	Tricosane	21632	25912	6799	21704	13977	25968	16536	20376	5716	2518	2916	8062	7523	23544	7318	13581	8456	22464	44896	19056	8033	75200	7830	28864	5248
	3 Me C23	170560	221248	78880	219264	61696	230912	175360	149952	85896	44448	32976	56920	28680	272832	89752	36440	145536	174848	357952	233280	74240	137856	113032	236672	90520
	Pentacosene	184256	198080	72616	184448	82632	175552	167104	146624	110912	54088	45824	65312	48280	202496	75328	61760	160576	205056	331264	226880	100560	200000	106176	167872	76528
	Pentacosane	2826	5469	1543	6233	2676	3247	3053	5039		589		1017	981	6522	2041	639	4533	3674	10860	5622	1546	10029	2871	6432	1533
	3 Me C25	7660	6163	3774	7822	2029	14140	8106	7619				1546		11406	7334	493	8957	8090	19160	13078	4496		7087	17760	6411
Abundance of M+1	Tricosane	4664	7567	1305	3942	3429	6549	4894	6854	2533	1460	802	1609	1347	7653	1826	2552	3132	2993	11897	4726	2638	15260	1557	6547	1537
	3 Me C23	35696	51472	20728	48032	13051	56680	32152	40736	14256	9304	10039	13520	9386	53912	17480	8348	26864	39672	73560	61072	16448	31976	27648	53760	20504
	Pentacosene	50152	60768	17296	48904	20168	48800	42248	35104	27232	14100	17520	18904	13184	57912	20360	15189	44016	49312	84368	56448	25400	52984	28552	45280	22768
	Pentacosane	1319	1478		1252	1581	2168	251	944		350		275	658	3926	443	623	678	1005	2041	1155	574	2084	395	2173	453
	3 Me C25	432	1731	794	2770	1148	4018	1842	3024				504		4102	1791		2254	1304	4228	1719	573		1737	5923	1211
Abundance of M+2	Tricosane	530	1272		568	327	1046	377	761				360		553		393		1241	1508	1550	593	1659	317	1365	370
	3 Me C23	3531	3502	2208	4435	1965	5939	3955	5498	1589	899	650	1923	676	3983	2712	561	3408	7724	7378	6398	1249	3988	2695	6787	1790
	Pentacosene	6727	5713	1560	5946	2244	5657	4449	4718	4442	2034	3411	2102	1647	9391	2972	1943	6940	3603	11501	8322	3602	8524	3932	6868	1654
	Pentacosane	176	613		302										578	150			192	1015			981		655	
	3 Me C25		278				713	182	245						230					420	1000			225	255	
Percentage of M+1	Tricosane	21.6	29.2	19.2	18.2	24.5	25.2	29.6	33.6	44.3	58.0	27.5	20.0	17.9	32.5	25.0	18.8	37.0	13.3	26.5	24.8	32.8	20.3	19.9	22.7	29.3
	3 Me C23	20.9	23.3	26.3	21.9	21.2	24.5	18.3	27.2	16.6	20.9	30.4	23.8	32.7	19.8	19.5	22.9	18.5	22.7	20.6	26.2	22.2	23.2	24.5	22.7	22.7
	Pentacosene	27.2	30.7	23.8	26.5	24.4	27.8	25.3	23.9	24.6	26.1	38.2	28.9	27.3	28.6	27.0	24.6	27.4	24.0	25.5	24.9	25.3	26.5	26.9	27.0	29.8
	Pentacosane	46.7	27.0	0.0	20.1	59.1	66.8	8.2	18.7		59.4		27.0	67.1	60.2	21.7	97.5	15.0	27.4	18.8	20.5	37.1	20.8	13.8	33.8	29.5
	3 Me C25	5.6	28.1	21.0	35.4	56.6	28.4	22.7	39.7				32.6		36.0	24.4	0.0	25.2	16.1	22.1	13.1	12.7		24.5	33.4	18.9
Percentage of M+2	Tricosane	2.5	4.9	0.0	2.6	2.3	4.0	2.3	3.7	0.0	0.0	0.0	4.5	0.0	2.3	0.0	2.9	0.0	5.5	3.4	8.1	7.4	2.2	4.0	4.7	7.1
	3 Me C23	2.1	1.6	2.8	2.0	3.2	2.6	2.3	3.7	1.8	2.0	2.0	3.4	2.4	1.5	3.0	1.5	2.3	4.4	2.1	2.7	1.7	2.9	2.4	2.9	2.0
	Pentacosene	3.7	2.9	2.1	3.2	2.7	3.2	2.7	3.2	4.0	3.8	7.4	3.2	3.4	4.6	3.9	3.1	4.3	1.8	3.5	3.7	3.6	4.3	3.7	4.1	2.2
	Pentacosane	6.2	11.2	0.0	4.8	0.0	0.0	0.0	0.0		0.0		0.0	0.0	8.9	7.3	0.0	0.0	5.2	9.3	0.0	0.0	9.8	0.0	10.2	0.0
	3 Me C25	0.0	4.5	0.0	0.0	0.0	5.0	2.2	3.2				0.0		2.0	0.0	0.0	0.0	0.0	2.2	7.6	0.0		3.2	1.4	0.0

0
0-20
20-40
40-60
60-80
80-100
100+

Figure B.29: Data sheet showing page one of the raw negative control data (propionic acid) for the propionate experiment for *Myrmica scabrinodis*.

Propionic Acid (Negative Control)		*Myrmica scabrinodis*																								
		Con 26	Con 27	Con 28	Con 29	Con 30	Con 31	Con 32	Con 33	Con 34	Con 35	Con 36	Con 37	Con 38	Con 39	Con 40	Con 41	Con 42	Con 43	Con 44	Con 45	Con 46	Con 47	Con 48	Con 49	Con 50
Abundance of M	Tricosane	17360	27072	3331	54168	109024	21384	11205	39384	5425	12145	3002	25792	18312	16576	13832	9992	4979	6785	13202	7772	8319	5266	15053	3453	4147
	3 Me C23	114776	153024	65768	332224	589824	169280	153920	227520	48688	72096	48480	178944	108392	139776	114328	113088	52576	95280	107496	122216	112840	88096	292352	94344	37392
	Pentacosene	116648	166080	56840	266368	527232	145216	127824	226752	36056	136320	54016	171200	195840	189120	89144	51496	76568	102168	98904	101352	101352	89208	231872	2675	25088
	Penatcosane	5909	4521	957	12378	22128	6506	3591	4359	1072	1542	850	2890	2698	2212	1826	611	1096	2805	3780	2733	1539	1209	2780	7100	743
	3 Me C25	5499	3619	4533	23024	19064	8957	9868	6059	1370	12444	3667	6913	2299	5148	1353	2580	3828	4716	2515	6826	5471	3448	21752	7839	2675
Abundance of M+1	Tricosane	3627	4531	596	10620	22984	6173	2132	9380	1019	3156	1575	4079	3644	4748	5186	3326	1509	3089	2695	1497	2763	1264	5352	839	1054
	3 Me C23	27992	36736	14217	72456	129600	34400	37752	45728	10096	11169	9031	37456	23832	28672	24472	25016	10719	26400	18784	26432	27352	24792	53768	21144	8250
	Pentacosene	34272	43184	19344	60584	139968	43056	32616	54416	9666	45192	10748	45280	50640	49032	32304	10458	20288	22568	26432	26344	28712	27536	68128	271	6366
	Penatcosane	1617	808	431	2778	5912	1807	1341	1330		587	326	227	1621	883	680		330	999	1007		737	261	984	2189	289
	3 Me C25	1003	1956	701	3422	2580	2489	1064	1720	506	3691	666	1938		1229	182	681	1576	986	1026	1475	1193	647	3860	1554	526
Abundance of M+2	Tricosane	1036	803		2244	3747	1441	388	1770	1129			412	483	649	540	769			334	195	394		288		
	3 Me C23	3510	3499	2180	7904	15686	4075	4479	3802	1483	997	1813	6218	3495	3768	2939	2775	2166		2711	2959	3298	3013	6422	2277	997
	Pentacosene	4395	4969	1944	8844	12287	4157	6901	10084	1151	3967	2534	3597	7741	3544	3682	1946	3426	2647	3640	4099	3490	3195	6641		691
	Penatcosane		169		403	710	227	673	322																240	
	3 Me C25			151	799	349	351	443	434		151								253	222	286				237	
Percentage of M+1	Tricosane	20.9	16.7	17.9	19.6	21.1	28.9	19.0	23.8	18.8	26.0	52.5	15.8	19.9	28.6	37.5	33.3	30.3	45.5	20.4	19.3	33.2	24.0	35.6	24.3	25.4
	3 Me C23	24.4	24.0	21.6	21.8	22.0	20.3	24.5	20.1	20.7	15.5	18.6	20.9	22.0	20.5	21.4	22.1	20.4	27.7	17.5	21.6	24.2	28.1	18.4	22.4	22.1
	Pentacosene	29.4	26.0	34.0	22.7	26.5	29.6	25.5	24.0	26.8	33.2	19.9	26.4	25.9	25.9	36.2	20.3	26.5	22.1	26.7	26.0	28.3	30.9	29.4	10.1	25.4
	Penatcosane	27.4	17.9	45.0	22.4	26.7	27.8	37.3	30.5	0.0	38.1	38.4	7.9	60.1	39.9	37.2	0.0	30.1	35.6	26.6	0.0	47.9	21.6	35.4	30.8	38.9
	3 Me C25	18.2	54.0	15.5	14.9	13.5	27.8	10.8	28.4	36.9	29.7	18.2	28.0	0.0	23.9	13.5	26.4	41.2	20.9	40.8	21.6	21.8	18.8	17.7	19.8	19.7
Percentage of M+2	Tricosane	6.0	3.0	0.0	4.1	3.4	6.7	3.5	4.5	20.8	0.0	0.0	1.6	2.6	3.9	3.9	7.7	0.0	0.0	2.5	2.5	4.7	0.0	1.9	0.0	0.0
	3 Me C23	3.1	2.3	3.3	2.4	2.7	2.4	2.9	1.7	3.0	1.4	3.7	3.5	3.2	2.7	2.6	2.5	4.1	0.0	2.5	2.4	2.9	3.4	2.2	2.4	2.7
	Pentacosene	3.8	3.0	3.4	3.3	2.3	2.9	5.4	4.4	3.2	2.9	4.7	2.1	4.0	1.9	4.1	3.8	4.5	2.6	3.7	4.0	3.4	3.6	2.9	0.0	2.8
	Penatcosane	0.0	3.7	0.0	3.3	3.2	3.5	18.7	7.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.4	0.0
	3 Me C25	0.0	0.0	3.3	3.5	1.8	3.9	4.5	7.2	0.0	1.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.4	8.8	4.2	0.0	0.0	0.0	3.0	0.0

0
0-20
20-40
40-60
60-80
80-100
100+

Figure B.30: Data sheet showing page two of the raw negative control data (propionic acid) for the propionate experiment for *Myrmica scabrinodis*.

Propionic Acid (Negative Control)		*Myrmica scabrinodis*							
		Con 51	Con 52	Con 53	Con 54	Con 55	Con 56	Con 57	Con 58
Abundance of M	Tricosane	12420	3824	8187	13611	5941	35600	9334	21536
	3 Me C23	54384	25248	48056	80648	35336	229248	928000	46000
	Pentacosene	43360	22208	44200	63192	34392	206528	98296	60040
	Pentacosane	3224	687	1859	5204	1347	12730	2775	1964
	3 Me C25	3123	838	3298	4834	1050	22824	1766	
Abundance of M+1	Tricosane	2695	1377	2082	3195	1612	7439	3801	3990
	3 Me C23	12397	5071	10703	18144	8247	50808	21784	8720
	Pentacosene	10811	6100	10882	15998	9592	55176	29072	17368
	Pentacosane	869	213	381	1613	365	3036	266	333
	3 Me C25	526		726	932	248	5120	309	
Abundance of M+2	Tricosane	319	169	555	487	230	820		797
	3 Me C23	1219	495	1232	1647	742	5578	2658	1241
	Pentacosene	1385	825	1219	2064	1010	7233	3865	2573
	Pentacosane						642		
	3 Me C25				154		520		
Percentage of M+1	Tricosane	21.7	36.0	25.4	23.5	27.1	20.9	40.7	18.5
	3 Me C23	22.8	20.1	22.3	22.5	23.3	22.2	2.3	19.0
	Pentacosene	24.9	27.5	24.6	25.3	27.9	26.7	29.6	28.9
	Pentacosane	27.0	31.0	20.5	31.0	27.1	23.8	9.6	17.0
	3 Me C25	16.8	0.0	22.0	19.3	23.6	22.4	17.5	
Percentage of M+2	Tricosane	2.6	4.4	6.8	3.6	3.9	2.3	0.0	3.7
	3 Me C23	2.2	2.0	2.6	2.0	2.1	2.4	0.3	2.7
	Pentacosene	3.2	3.7	2.8	3.3	2.9	3.5	3.9	4.3
	Pentacosane	0.0	0.0	0.0	0.0	0.0	5.0	0.0	0.0
	3 Me C25	0.0	0.0	0.0	3.2	0.0	2.3	0.0	

0
0-20
20-40
40-60
60-80
80-100
100+

Figure B.31: Data sheet showing page three of the raw negative control data (propionic acid) for the propionate experiment for *Myrmica scabrinodis*.

Sodium [1-^{13}C]acetate (Positive Control)		*Myrmica scabrinodis*																								
		Con 1	Con 2	Con 3	Con 4	Con 5	Con 6	Con 7	Con 8	Con 9	Con 10	Con 11	Con 12	Con 13	Con 14	Con 15	Con 16	Con 17	Con 18	Con 19	Con 20	Con 21	Con 22	Con 23	Con 24	Con 25
Abundance of M	Tricosane	4332	5445	5285	2635	2745	3768	1890	4107	2595	2639	793	2609	2012	4086	4649	13056	3141	3010	5829	6495	8316	4534	5650	3323	1450
	3 Me C23	34888	66752	70072	31040	23472	32488	24920	36344	28888	19616	7690	14061	13436	41704	33160	58336	28720	26392	26664	41872	31104	35424	31856	28808	15657
	Pentacosene	30336	49832	50816	20656	18776	26840	18920	31608	22424	17904	6411	14145	11721	31360	27048	49456	25920	23632	22088	39488	27336	31720	28904	23304	13756
	Pentacosane	649	778	919	466	791	608	323	872	521	493		410	384	704	965	2840	659	422	1815	1347	3250	958	1062	725	188
	3 Me C25	785	3454	4288	1487	694	1676	963	865	1017	691		375	595	2177	1350	947	825	926	1496	1807	2803	1176	1471	1644	783
Abundance of M+1	Tricosane	2420	2384	2115	1106	1515	1479	891	2083	1325	1358	587	1356	997	1988	1912	4466	901	1184	2087	2081	3037	1918	2038	1008	753
	3 Me C23	17208	20288	21896	10218	11047	11488	8226	14776	9949	9044	3963	6432	5605	15799	13224	21904	9134	9451	9122	15589	11856	14515	12175	8623	7995
	Pentacosene	12739	16464	17936	8197	8671	10200	7135	14076	7737	7579	3278	6146	4335	12565	11051	18056	8554	9437	8174	14438	10854	13905	10515	7861	6518
	Pentacosane	469	350	437	256	309	422		461	245	181		161	267	429	562	1432	268	261	421	617	1102	426	458	231	
	3 Me C25	449	1143	1265	532	417	716	302	533	557	422		256	165	823	637	468	266	355	529	496	782	562	643	523	180
Abundance of M+2	Tricosane	995	776	792	395	718	472	246	759	353	586	339	408	272	468	652	902	312	268	390	587	600	532	476	244	431
	3 Me C23	8467	6283	6119	3248	5349	3930	2611	6438	3664	3026	2385	3002	2411	6013	5232	7137	3222	3386	2555	5166	4540	5553	4214	2378	3790
	Pentacosene	4099	4153	4018	1947	2877	2570	1457	3681	1919	2302	1140	1722	1253	2856	2894	4655	1911	2047	1799	3579	2571	3585	2773	1761	2333
	Pentacosane	226		193	194	199			200						184					213	229	173	153			
	3 Me C25	296	331	343	168		244		215	171	222				369	378					180	281	249	240	151	178
Percentage of M+1	Tricosane	55.9	43.8	40.0	42.0	55.2	39.3	47.1	50.7	51.1	51.5	74.0	52.0	49.6	48.7	41.1	34.2	28.7	39.3	35.8	32.0	36.5	42.3	36.1	30.3	51.9
	3 Me C23	49.3	30.4	31.2	32.9	47.1	35.4	33.0	40.7	34.4	46.1	51.5	45.7	41.7	37.9	39.9	37.5	31.8	35.8	34.2	37.2	38.1	41.0	38.2	29.9	51.1
	Pentacosene	42.0	33.0	35.3	39.7	46.2	38.0	37.7	44.5	34.5	42.3	51.1	43.4	37.0	40.1	40.9	36.5	33.0	39.9	37.0	36.6	39.7	43.8	36.4	33.7	47.4
	Pentacosane	72.3	45.0	47.6	54.9	39.1	69.4	0.0	52.9	47.0	36.7		39.3	69.5	60.9	58.2	50.4	40.7	61.8	23.2	45.8	33.9	44.5	43.1	31.9	0.0
	3 Me C25	57.2	33.1	29.5	35.8	60.1	42.7	31.4	61.6	54.8	61.1		68.3	27.7	37.8	47.2	49.4	32.2	38.3	35.4	27.4	27.9	47.8	43.7	31.8	23.0
Percentage of M+2	Tricosane	23.0	14.3	15.0	15.0	26.2	12.5	13.0	18.5	13.6	22.2	42.7	15.6	13.5	11.5	14.0	6.9	9.9	8.9	6.7	9.0	7.2	11.7	8.4	7.3	29.7
	3 Me C23	24.3	9.4	8.7	10.5	22.8	12.1	10.5	17.7	12.7	15.4	31.0	21.3	17.9	14.4	15.8	12.2	11.2	12.8	9.6	12.3	14.6	15.7	13.2	8.3	24.2
	Pentacosene	13.5	8.3	7.9	9.4	15.3	9.6	7.7	11.6	8.6	12.9	17.8	12.2	10.7	9.1	10.7	9.4	7.4	8.7	8.1	9.1	9.4	11.3	9.6	7.6	17.0
	Pentacosane	34.8	0.0	21.0	41.6	25.2	0.0	0.0	22.9	0.0	0.0		0.0	0.0	26.1	0.0	0.0	0.0	0.0	11.7	17.0	5.3	16.0	0.0	0.0	0.0
	3 Me C25	37.7	9.6	8.0	11.3	0.0	14.6	0.0	24.9	16.8	32.1		0.0	0.0	16.9	28.0	0.0	0.0	0.0	0.0	10.0	10.0	21.2	16.3	9.2	22.7

0
0-20
20-40
40-60
60-80
80-100
100+

Figure B.32: Data sheet showing page one of the raw positive control data (sodium [1-^{13}C]acetate) for the propionate experiment for *Myrmica scabrinodis*.

Sodium [1-^{13}C]acetate (Positive Control)		*Myrmica scabrinodis*																								
		Con 26	Con 27	Con 28	Con 29	Con 30	Con 31	Con 32	Con 33	Con 34	Con 35	Con 36	Con 37	Con 38	Con 39	Con 40	Con 41	Con 42	Con 43	Con 44	Con 45	Con 46	Con 47	Con 48	Con 49	Con 50
Abundance of M	Tricosane	4454	2375	3148	5401	4259	11289	5248	2174	5054	4263	5167	11749	20768	6137	7046	6336	3516	738	2473	8970	3244	23976	4248	8977	6197
	3 Me C23	42208	29672	31736	28496	37720	23144	50552	24984	47600	39136	36936	36624	85984	55800	31000	23504	26976	6774	23032	43096	21488	126728	32024	25840	31864
	Pentacosene	35224	22480	24016	25656	31104	18888	40208	21200	37384	33928	31496	43720	114736	52328	34552	32120	30360	7090	20120	38056	29608	121128	27664	18040	24088
	Pentacosane	715	666	732	1208	1083	3639	1216	488	1400	783	946	2531	4613	1140	1127	1041	561	299	628	2142	500	5569	1336	2495	1978
	3 Me C25	1739	2404	2196	730	1957	2325	3289	1015	2736	1623	1611	1511	3894	2620	923	810	696	190	1263	1945	447	5255	1387	2016	1878
Abundance of M+1	Tricosane	2141	981	917	2189	2317	3738	2526	1101	2368	1973	1777	5186	8535	2333	2968	2761	1611	468	1008	3717	1094	7960	1583	3448	2040
	3 Me C23	16808	8762	9159	10366	19472	12174	19928	12322	17288	14987	12976	16364	34624	18808	13411	10911	13685	3533	6849	14926	10796	48368	11962	9729	11309
	Pentacosene	14650	8011	7557	9489	15771	9643	15443	9595	15019	13619	11424	16592	43536	17512	13765	14051	12978	3816	6888	13587	13632	47880	10818	7926	8649
	Pentacosane	436	299	228	437	428	1175	460	273	550	381	448	1070	1731	541	513	646	199		291	912	271	2005	469	804	748
	3 Me C25	761	743	534	348	1251	790	1487	625	1049	838	552	597	1248	809	456	486	502	153	264	726	342	2069	354	596	632
Abundance of M+2	Tricosane	462	317	237	751	1243	1206	823	416	713	549	660	1567	2233	684	1004	862	640	260	228	910	820	2572	486	606	428
	3 Me C23	6986	1958	2041	3513	9849	5923	7166	6168	5907	5469	4046	5995	13841	5411	5318	5282	6334	2208	2076	5174	4823	17784	4247	3872	2763
	Pentacosene	3552	1421	1435	2467	5824	3342	4055	3253	3642	3701	2816	5047	12517	4018	3659	3848	4288	1491	1514	3930	3329	12687	2514	1822	1652
	Pentacosane	179				195	259	243		190		184	245	607	183	252	245				182		561		194	156
	3 Me C25	534				496	317	457	257	198	246	158	288	601	219	159		186			232	276	630	162	299	
Percentage of M+1	Tricosane	48.1	41.3	29.1	40.5	54.4	33.1	48.1	50.6	46.9	46.3	34.4	44.1	41.1	38.0	42.1	43.6	45.8	63.4	40.8	41.4	33.7	33.2	37.3	38.4	32.9
	3 Me C23	39.8	29.5	28.9	36.4	51.6	52.6	39.4	49.3	36.3	38.3	35.1	44.7	40.3	33.7	43.3	46.4	50.7	52.2	29.7	34.6	50.2	38.2	37.4	37.7	35.5
	Pentacosene	41.6	35.6	31.5	37.0	50.7	51.1	38.4	45.3	40.2	40.1	36.3	38.0	37.9	33.5	39.8	43.7	42.7	53.8	34.2	35.7	46.0	39.5	39.1	43.9	35.9
	Pentacosane	61.0	44.9	31.1	36.2	39.5	32.3	37.8	55.9	39.3	48.7	47.4	42.3	37.5	47.5	45.5	62.1	35.5	0.0	46.3	42.6	54.2	36.0	35.1	32.2	37.8
	3 Me C25	43.8	30.9	24.3	47.7	63.9	34.0	45.2	61.6	38.3	51.6	34.3	39.5	32.0	30.9	49.4	60.0	72.1	80.5	20.9	37.3	76.5	39.4	25.5	29.6	33.7
Percentage of M+2	Tricosane	10.4	13.3	7.5	13.9	29.2	10.7	15.7	19.1	14.1	12.9	12.8	13.3	10.8	11.1	14.2	13.6	18.2	35.2	9.2	10.1	25.3	10.7	11.4	6.8	6.9
	3 Me C23	16.6	6.6	6.4	12.3	26.1	25.6	14.2	24.7	12.4	14.0	11.0	16.4	16.1	9.7	17.2	22.5	23.5	32.6	9.0	12.0	22.4	14.0	13.3	15.0	8.7
	Pentacosene	10.1	6.3	6.0	9.6	18.7	17.7	10.1	15.3	9.7	10.9	8.9	11.5	10.9	7.7	10.6	12.0	14.1	21.0	7.5	10.3	11.2	10.5	9.1	10.1	6.9
	Pentacosane	25.0	0.0	0.0	0.0	18.0	7.1	20.0	0.0	13.6	0.0	19.5	9.7	13.2	16.1	22.4	23.5	0.0	0.0	0.0	8.5	0.0	10.1	0.0	7.8	7.9
	3 Me C25	30.7	0.0	0.0	0.0	25.3	13.6	13.9	25.3	7.2	15.2	9.8	19.1	15.4	8.4	17.2	0.0	26.7	0.0	0.0	11.9	61.7	12.0	11.7	14.8	0.0

0
0-20
20-40
40-60
60-80
80-100
100+

Figure B.33: Data sheet showing page two of the raw positive control data (sodium [1-^{13}C]acetate) for the propionate experiment for *Myrmica scabrinodis*.

Sodium [1-^{13}C]acetate (Positive Control)		*Myrmica scabrinodis*										
		Con 51	Con 52	Con 53	Con 54	Con 55	Con 56	Con 57	Con 58	Con 59	Con 60	Con 61
Abundance of M	Tricosane	8532	3062	8559	3597	3957	4450	2218	4639	8527	3700	4048
	3 Me C23	23616	19232	22144	33512	35208	23984	25792	25592	26200	37064	27560
	Pentacosene	20784	17440	20744	28488	28736	24888	20000	22592	22816	29768	22472
	Pentacosane	2825	759	2172	670	620	1111	524	861	2297	856	928
	3 Me C25	1177	622	901	2046	2252	1337	1138	1160	1498	1145	671
Abundance of M+1	Tricosane	3278	1877	2509	1992	2202	1576	1622	1941	3319	2044	1724
	3 Me C23	10620	10290	6625	16206	16308	8160	12718	12663	12349	15153	11598
	Pentacosene	8804	7685	7844	12473	11946	9075	9262	9480	10625	13079	9462
	Pentacosane	923	358	785	525	376	570	388	744	878	495	300
	3 Me C25	730	393	361	1133	991	503	795	577	657	877	322
Abundance of M+2	Tricosane	1127	511	511	472	635	442	697	652	982	876	580
	3 Me C23	4667	4906	2306		7354	2907	6323	5826	6275	6481	4846
	Pentacosene	2425	2540	1522	3777	3004	2155	3006	2741	3080	4021	2787
	Pentacosane	233	232	158		283	275	157		242	159	
	3 Me C25		237		503	421	262	279	260	234	259	202
Percentage of M+1	Tricosane	38.4	61.3	29.3	55.4	55.6	35.4	73.1	41.8	38.9	55.2	42.6
	3 Me C23	45.0	53.5	29.9	48.4	46.3	34.0	49.3	49.5	47.1	40.9	42.1
	Pentacosene	42.4	44.1	37.8	43.8	41.6	36.5	46.3	42.0	46.6	43.9	42.1
	Pentacosane	32.7	47.2	36.1	78.4	60.6	51.3	74.0	86.4	38.2	57.8	32.3
	3 Me C25	62.0	63.2	40.1	55.4	44.0	37.6	69.9	49.7	43.9	76.6	48.0
Percentage of M+2	Tricosane	13.2	16.7	6.0	13.1	16.0	9.9	31.4	14.1	11.5	23.7	14.3
	3 Me C23	19.8	25.5	10.4	0.0	20.9	12.1	24.5	22.8	24.0	17.5	17.6
	Pentacosene	11.7	14.6	7.3	13.3	10.5	8.7	15.0	12.1	13.5	13.5	12.4
	Pentacosane	8.2	30.6	7.3	0.0	45.6	24.8	30.0	0.0	10.5	18.6	0.0
	3 Me C25	0.0	38.1	0.0	24.6	18.7	19.6	24.5	22.4	15.6	22.6	30.1

0
0-20
20-40
40-60
60-80
80-100
100+

Figure B.34: Data sheet showing page three of the raw positive control data (sodium [1-^{13}C]acetate) for the propionate experiment for *Myrmica scabrinodis*.

Propionic-2,2-d_2 acid-d (Substrate)		*Myrmica scabrinodis*																								
		Sub 1	Sub 2	Sub 3	Sub 4	Sub 5	Sub 6	Sub 7	Sub 8	Sub 9	Sub 10	Sub 11	Sub 12	Sub 13	Sub 14	Sub 15	Sub 16	Sub 17	Sub 18	Sub 19	Sub 20	Sub 21	Sub 22	Sub 23	Sub 24	Sub 25
Abundance of M	Tricosane	8662	8360	3626	8168	2853	1518	5973	6094	4240	5802	7875	2578	5585	4477	4298	9551	8448	5539	7092	3511	5003	9186	7401	16342	5040
	3 Me C23	14182	45152	15039	33336	17112	8596	28688	26928	16080	11980	35560	19224	25336	18296	15022	28184	32040	26048	52128	37888	10037	87872	11184	123368	44120
	Pentacosene	13121	51280	19336	35264	19800	10990	32712	29168	18560	16028	42728	19704	20432	17912	17752	30040	28512	26616	49144	30864	12586	67232	12621	104776	36240
	Pentacosane	1868	1422	529	1342	661	279	865	850	768	489	11534	401	835	521	459	1242	1727	1054	1267	738	538	2638	1010	4826	853
	3 Me C25	798	1536	564	588	474		918	496	210		667	555	328	245	155	353	670	577	1595	1947	384	6705	284	8560	1683
Abundance of M+1	Tricosane	3414	1880	996	2282	684	426	1516	1291	1083	1412	2315	633	1681	1322	1107	2440	1940	1469	1779	998	1310	2214	1790	4047	1525
	3 Me C23	4056	11176	3988	7425	4183	2050	6990	6695	4423	2824	8653	4871	5733	4453	3345	6580	7375	6224	12471	8909	2912	20232	2815	28840	10102
	Pentacosene	4290	12926	6128	9379	5536	3112	7925	7509	4752	4469	10818	5631	5634	5276	4564	8791	7819	7177	12925	8555	3658	17104	3670	28600	9683
	Pentacosane	758	527	274	398	202		354	318	291	155	583	172	237	152	194	327	563	250	374	248	179	683	371	1407	259
	3 Me C25	313	345	150	286			246	190			238	242	180			197	156	158	360	521	152	1654		2159	479
Abundance of M+2	Tricosane	644	364		375			186	242	188	212	305					369	299	172	237		171	305	244	619	201
	3 Me C23	383	1532	658	871	481	254	923	874	468	264	1036	618	786	442	458	695	944	742	1135	961	276	1993	346	2954	1211
	Pentacosene	774	1825	649	1167	795	312	1302	1220	740	749	1445	824	696	629	539	1223	918	863	1921	1263	470	2625	477	3433	1403
	Pentacosane																								182	
	3 Me C25																			202			252		199	
Percentage of M+1	Tricosane	39.4	22.5	27.5	27.9	24.0	28.1	25.4	21.2	25.5	24.3	29.4	24.6	30.1	29.5	25.8	25.5	23.0	26.5	25.1	28.4	26.2	24.1	24.2	24.8	30.3
	3 Me C23	28.6	24.8	26.5	22.3	24.4	23.8	24.4	24.9	27.5	23.6	24.3	25.3	22.6	24.3	22.3	23.3	23.0	23.9	23.9	23.5	29.0	23.0	25.2	23.4	22.9
	Pentacosene	32.7	25.2	31.7	26.6	28.0	28.3	24.2	25.7	25.6	27.9	25.3	28.6	27.6	29.5	25.7	29.3	27.4	27.0	26.3	27.7	29.1	25.4	29.1	27.3	26.7
	Pentacosane	40.6	37.1	51.8	29.7	30.6	0.0	40.9	37.4	37.9	31.7	5.1	42.9	28.4	29.2	42.3	26.3	32.6	23.7	29.5	33.6	33.3	25.9	36.7	29.2	30.4
	3 Me C25	39.2	22.5	26.6	48.6	0.0		26.8	38.3	0.0		35.7	43.6	54.9	0.0	0.0	55.8	23.3	27.4	22.6	26.8	39.6	24.7	0.0	25.2	28.5
Percentage of M+2	Tricosane	7.4	4.4	0.0	4.6	0.0	0.0	3.1	4.0	4.4	3.7	3.9	0.0	0.0	0.0	0.0	3.9	3.5	3.1	3.3	0.0	3.4	3.3	3.3	3.8	4.0
	3 Me C23	2.7	3.4	4.4	2.6	2.8	3.0	3.2	3.2	2.9	2.2	2.9	3.2	3.1	2.4	3.0	2.5	2.9	2.8	2.2	2.5	2.7	2.3	3.1	2.4	2.7
	Pentacosene	5.9	3.6	3.4	3.3	4.0	2.8	4.0	4.2	4.0	4.7	3.4	4.2	3.4	3.5	3.0	4.1	3.2	3.2	3.9	4.1	3.7	3.9	3.8	3.3	3.9
	Pentacosane	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.8	0.0
	3 Me C25	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	12.7	0.0	0.0	3.8	0.0	2.3	0.0

0
0-20
20-40
40-60
60-80
80-100
100+

Figure B.35: Data sheet showing page one of the raw substrate data (propionic-2,2-d_2 acid-d) for the propionate experiment for *Myrmica scabrinodis*.

Propionic-2,2-d_2 acid-d (Substrate)		*Myrmica scabrinodis*																								
		Sub 26	Sub 27	Sub 28	Sub 29	Sub 30	Sub 31	Sub 32	Sub 33	Sub 34	Sub 35	Sub 36	Sub 37	Sub 38	Sub 39	Sub 40	Sub 41	Sub 42	Sub 43	Sub 44	Sub 45	Sub 46	Sub 47	Sub 48	Sub 49	Sub 50
Abundance of M	Tricosane	7031	5522	4991	8511	9087	7421	4187	7523	2613	4095	5224	4216	7650	4622	4306	5003	4623	5428	7465	6063	6642	4101	6601	3099	5206
	3 Me C23	40120	29456	25776	89432	62768	48856	41232	57400	27704	27848	30928	45184	31528	44440	37280	44880	26768	39768	56520	37504	32656	31288	42696	24312	33056
	Pentacosene	39280	33072	28712	60296	54032	52592	33200	44672	20360	22560	30216	31120	20768	32104	27128	33312	24520	28136	36384	33152	27528	29640	32016	19632	27944
	Pentacosane	2687	884	634	1971	1866	1378	1014	1113	385	808	1227	1028	3060	1019	902	1271	1203	1522	3169	1743	2002	1367	1645	933	1839
	3 Me C25	1442	1107	1024	6354	3612	3050	2080	2360	970	954	2881	6829	6848	7209	6130	6569	2106	7230	10783	5190	4765	2919	6133	2650	3767
Abundance of M+1	Tricosane	2353	1534	1311	2270	2251	1505	1306	1517	703	1084	1336	1392	1880	1464	1162	1117	1460	1335	2031	1395	1722	1162	1728	952	1660
	3 Me C23	12594	6888	6004	20344	13176	11865	9471	13145	5612	6772	7376	10418	7389	10171	8956	10775	6054	9963	12390	9021	7781	8369	10076	5576	7853
	Pentacosene	12856	9555	7726	14941	14298	13494	8743	11845	6076	6805	8450	8013	5705	8122	7959	8804	6924	7387	9555	8117	7281	8112	8851	5817	8161
	Pentacosane	552	290	227	665	597	374	261	309		277	276	442	658	418	176	358	387	610	606	579	572	364	464	207	657
	3 Me C25	534	347	267	1538	876	662	556	816	297	323	594	1898	1855	2245	1618	1960	509	2317	2630	1347	1243	772	1545		1215
Abundance of M+2	Tricosane	403		207	362	348	209	222	192	176	169	306	174	259	165		195		158	256	217	255	160	224		197
	3 Me C23	3697	985	918	2319	1861	1301	1096	1538	665	612	1037	1247	311	1289	893	967	759	1369	1580	944	1290	1091	1393	848	991
	Pentacosene	2687	1212	1163	2323	1985	1789	919	1467	687	824	1055	922	524	1007	1044	1473	921	1021	1369	1166	1075	885	1175	794	830
	Pentacosane	191												152												
	3 Me C25				180							165	267	193	334	242	233		357	381	209	161		208	738	
Percentage of M+1	Tricosane	33.5	27.8	26.3	26.7	24.8	20.3	31.2	20.2	26.9	26.5	25.6	33.0	24.6	31.7	27.0	22.3	31.6	24.6	27.2	23.0	25.9	28.3	26.2	30.7	31.9
	3 Me C23	31.4	23.4	23.3	22.7	21.0	24.3	23.0	22.9	20.3	24.3	23.8	23.1	23.4	22.9	24.0	24.0	22.6	25.1	21.9	24.1	23.8	26.7	23.6	22.9	23.8
	Pentacosene	32.7	28.9	26.9	24.8	26.5	25.7	26.3	26.5	29.8	30.2	28.0	25.7	27.5	25.3	29.3	26.4	28.2	26.3	26.3	24.5	26.4	27.4	27.6	29.6	29.2
	Pentacosane	20.5	32.8	35.8	33.7	32.0	27.1	25.7	27.8	0.0	34.3	22.5	43.0	21.5	41.0	19.5	28.2	32.2	40.1	19.1	33.2	28.6	26.6	28.2	22.2	35.7
	3 Me C25	37.0	31.3	26.1	24.2	24.3	21.7	26.7	34.6	30.6	33.9	20.6	27.8	27.1	31.1	26.4	29.8	24.2	32.0	24.4	26.0	26.1	26.4	25.2	0.0	32.3
Percentage of M+2	Tricosane	5.7	0.0	4.1	4.3	3.8	2.8	5.3	2.6	6.7	4.1	5.9	4.1	3.4	3.6	0.0	3.9	0.0	2.9	3.4	3.6	3.8	3.9	3.4	0.0	3.8
	3 Me C23	9.2	3.3	3.6	2.6	3.0	2.7	2.7	2.7	2.4	2.2	3.4	2.8	1.0	2.9	2.4	2.2	2.8	3.4	2.8	2.5	4.0	3.5	3.3	3.5	3.0
	Pentacosene	6.8	3.7	4.1	3.9	3.7	3.4	2.8	3.3	3.4	3.7	3.5	3.0	2.5	3.1	3.8	4.4	3.8	3.6	3.8	3.5	3.9	3.0	3.7	4.0	3.0
	Pentacosane	7.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	3 Me C25	0.0	0.0	0.0	2.8	0.0	0.0	0.0	0.0	0.0	0.0	5.7	3.9	2.8	4.6	3.9	3.5	0.0	4.9	3.5	4.0	3.4	0.0	3.4	27.8	0.0

0
0-20
20-40
40-60
60-80
80-100
100+

Figure B.36: Data sheet showing page two of the raw substrate data (propionic-2,2-d_2 acid-d) for the propionate experiment for *Myrmica scabrinodis*.

Appendix C

Excel data sheets of the raw data for Chapter 5

The following pages show the raw data sheets from the amino acid substrate experiments. The cells are colour coded according to the percentage abundance calculated from the raw mass spectra data, see Section 3.4.2.1. Note that there was no usable substrate data obtained for *Formica lemani* and L-valine $^{13}C_5$,^{15}N, therefore only the control data is shown for this part of the experiment. Due to the size of these experiments and the number of samples in some cases the data extends across several pages.

Amino Acid Experiment

Figure C.1-C.2: Raw control data for *Formica lemani* and L-leucine 5,5,5-d_3
Figure C.3-C.4: Raw substrate data for *Formica lemani* and L-leucine 5,5,5-d_3
Figure C.5-C.6: Raw control data for *Myrmica sabuleti* and L-leucine 5,5,5-d_3
Figure C.7-C.8: Raw substrate data for *Myrmica sabuleti* and L-leucine 5,5,5-d_3

Figure C.9-C.10: Raw control data for *Formica lemani* and L-leucine $^{13}C_6$,^{15}N
Figure C.11-C.14: Raw substrate data for *Formica lemani* and L-leucine $^{13}C_6$,^{15}N
Figure C.15-C.16: Raw control data for *Myrmica sabuleti* and L-leucine $^{13}C_6$,^{15}N
Figure C.17-C.21: Raw substrate data for *Myrmica sabuleti* and L-leucine $^{13}C_6$,^{15}N

Figure C.22-C.25: Raw control data for *Formica lemani* and DL-valine d_8
Figure C.26-C.29: Raw substrate data for *Formica lemani* and DL-valine d_8
Figure C.30-C.33: Raw control data for *Myrmica sabuleti* and DL-valine d_8
Figure C.34-C.35: Raw substrate data for *Myrmica sabuleti* and DL-valine d_8

Figure C.36-C.37: Raw control data for *Formica lemani* and L-valine $^{13}C_5$,^{15}N
Figure C.38-C.39: Raw control data for *Myrmica sabuleti* and L-valine $^{13}C_5$,^{15}N
Figure C.40-C.44: Raw substrate data for *Myrmica sabuleti* and L-valine $^{13}C_5$,^{15}N

Figure C.45-C.46: Raw control data for *Formica lemani* and sodium [1-^{13}C]acetate

Figure C.47-C.48: Raw control data for *Myrmica sabuleti* and sodium [1-^{13}C]acetate

L-Leucine (Negative Control)		*Formica lemani*																								
		Con 1	Con 2	Con 3	Con 4	Con 5	Con 6	Con 7	Con 8	Con 9	Con 10	Con 11	Con 12	Con 13	Con 14	Con 15	Con 16	Con 17	Con 18	Con 19	Con 20	Con 21	Con 22	Con 23	Con 24	Con 25
Abundance of M	Tricosene	14728	21280	4192	15073	20640	19016	19312	4376	4680	7453	9553	20224	26464	7773	11123	6935	8241		6917	3721	10053	9500	10303	5691	23664
	Tricosane	5902	6298	1882	2658	5868	3137	1189	2800	840	3136	3994	4359	7118	2736	5097	3172	3849		1824	3280	2560	3788	4962	1399	2872
	3 Me C23	42536	68024	6499	27816	38784	27848	25232	8671	5856	23680	23936	38120	40848	14863	24232	23200	26424		13213	15260	11752	14962	26888	4705	30864
	Pentacosene	296064	849152	75704	366464	526976	410048	425344	105208	90704	283712	258240	429952	764928	183872	290752	250176	243392		156544	132224	249984	224896	320384	84424	428352
	Pentacosane	15441	16338	3821	10021	11538	11369	11943	12890	5386	16005	7132	11172	10746	3176	9355	7112	5468		4196	3151	3695	9355	11547	3739	8254
	3 Me C25	160640	199360	22632	103832	107672	90424	94064	27128	28208	65448	66624	98672	152064	50528	71968	55808	76984		42400	42640	66168	49960	79720	18152	103752
	Heptacosene	61368	162560	23064	83624	116504	89872	100952	25032	23688	72904	57504	90696	142016	43056	63952	57080	64080		45088	31184	61568	63560	68440	24408	81360
	Heptacosane	1210	6217	737	1443	2761	2038	854	4567	1557	1211	503	2255	3330	662	718	2140	528			247	831	2778	1107	575	1311
Abundance of M+1	Tricosene	6378	5652		6673	6147	7112	3335	1002	554	1835	2024	5525	7176	1704	2964	1288	3026		2395	1531	4025	1355	3350	1610	6629
	Tricosane	2538	2922		1185	2418	1301		359	1309	1427	1171	3448	3165	396	210		311		715	537	255	1655	1899		1689
	3 Me C23	9639	16047	1105	8634	8189	6104	7701	1963	572	6287	4905	8973	6676	3969	7578	4531	5063		3074	3436	3431	2462	7410	1700	7773
	Pentacosene	81584	209536	24600	104488	138816	106976	97904	26112	23328	63032	59728	116064	202752	41496	78432	59408	64632		40512	30248	66512	63216	93160	20672	119584
	Pentacosane	4148	5037	1637	2173	2479	4328	2359	4716	874	952	3093	2704	1618	734	878	2474	907		441	1256	2220	485	1512	764	3513
	3 Me C25	33720	46616	7370	22680	29000	24376	20192	9025	5886	1948	14328	23568	28608	6251	19176	19664	15616		13849	7927	14117	16160	24488	4868	23720
	Heptacosene	12312	40400	3286	26704	33008	28864	28968	8971	7488	23960	19392	22328	38824	13411	21464	21072	22216		10669	9656	21648	20184	25120	6269	24984
	Heptacosane	198	2100		571	681	1939		796	286	154			2018		857		390					1043	229		487
Abundance of M+2	Tricosene	1729	370		720	606	1304	3352					898	789		387		524					331	599		
	Tricosane	1377			509	277										278						410				
	3 Me C23	1189	1897		1568	1136	2837	435		439	852		624	1218	802	785	382	636					8648	792		1479
	Pentacosene	12098	790	1422	15753	21656	13419	12053	5323	3741	8227	10531	10456	26368	6471	8663	9920	7261		5905	4068	9723		13062	4695	11989
	Pentacosane	1432	570		328	205	778		1087		507	164		630	154		165									
	3 Me C25	2099	6219	2763	3621	4224	3306	1852	353	1326	1250	1201	793	4802	804	454	2204	1149		1577	1766	2013	853	4164	917	3222
	Heptacosene	4988	5825	1278	3929	4336	3375	2100	222	615	1453	2857	2914	4956	1582	1621	2515	1141		1377	3263	1557	3105	2699	605	3709
	Heptacosane																									
Abundance of M+3	Tricosene																	314								
	Tricosane	839																								
	3 Me C23																	155					869			
	Pentacosene	3154		881	1008	1262	1770	1872			2283	632	1246	1875	1916		155	343				480		1282		1496
	Pentacosane																280									
	3 Me C25	1590	1472	186		657	1687							401		534	354	590		822			712			
	Heptacosene	562	346			423	888	581			428					571						790	820			689
	Heptacosane																									

Figure C.1: Data sheet showing page one of the raw control data for the L-leucine 5,5,5-d_3 experiment and *Formica lemani*.

L-Leucine (Negative Control)		*Formica lemani*																							
		Con 1	Con 2	Con 3	Con 4	Con 5	Con 6	Con 7	Con 8	Con 9	Con 10	Con 11	Con 12	Con 13	Con 14	Con 15	Con 16	Con 17	Con 18	Con 19	Con 20	Con 21	Con 22	Con 23	Con 24	Con 25
Percentage of M+1	Tricosene	43.3	26.6	0.0	44.3	29.8	37.4	17.3	22.9	11.8	24.6	21.2	27.3	27.1	21.9	26.6	18.6	36.7		34.6	41.1	40.0	14.3	32.5	28.3	28.0
	Tricosane	43.0	46.4	0.0	44.6	41.2	41.5	0.0	12.8	155.8	45.5	29.3	79.1	44.5	14.5	4.1	0.0	8.1		39.2	16.4	10.0	43.7	38.3	0.0	58.8
	3 Me C23	22.7	23.6	17.0	31.0	21.1	21.9	30.5	22.6	9.8	26.5	20.5	23.5	16.3	26.7	31.3	19.5	19.2		23.3	22.5	29.2	16.5	27.6	36.1	25.2
	Pentacosene	27.6	24.7	32.5	28.5	26.3	26.1	23.0	24.8	25.7	22.2	23.1	27.0	26.5	22.6	27.0	23.7	26.6		25.9	22.9	26.6	28.1	29.1	24.5	27.9
	Pentacosane	26.9	30.8	42.8	21.7	21.5	38.1	19.8	36.6	16.2	5.9	43.4	24.2	15.1	23.1	9.4	34.8	16.6		10.5	39.9	60.1	5.2	13.1	20.4	42.6
	3 Me C25	21.0	23.4	32.6	21.8	26.9	27.0	21.5	33.3	20.9	3.0	21.5	23.9	18.8	12.4	26.6	35.2	20.3		32.7	18.6	21.3	32.3	30.7	26.8	22.9
	Heptacosene	20.1	24.9	14.2	31.9	28.3	32.1	28.7	35.8	31.6	32.9	33.7	24.6	27.3	31.1	33.6	36.9	34.7		23.7	31.0	35.2	31.8	36.7	25.7	30.7
	Heptacosane	16.4	33.8	0.0	39.6	24.7	95.1	0.0	17.4	18.4	12.7	0.0	0.0	60.6	0.0	119.4	0.0	73.9			0.0	0.0	37.5	20.7	0.0	37.1
Percentage of M+2	Tricosene	9.3	0.0	0.0	3.4	1.3	0.0	0.0	0.0	0.0	0.0	0.0	4.4	3.0	0.0	3.5	0.0	6.4		0.0	0.0	0.0	3.5	5.8	0.0	0.0
	Tricosane	20.1	30.1	0.0	59.0	19.4	90.4	36.6	0.0	52.3	0.0	0.0	0.0	0.0	0.0	5.5	0.0	0.0		0.0	0.0	16.0	0.0	0.0	0.0	0.0
	3 Me C23	28.4	1.2	21.9	56.6	55.8	48.2	47.8	61.4	63.9	3.6	0.0	1.6	3.0	5.4	3.2	1.6	2.4		0.0	0.0	0.0	57.8	2.9	0.0	4.8
	Pentacosene	0.5	0.1	0.0	0.1	0.0	0.2	0.0	1.0	0.0	2.9	4.1	2.4	3.4	3.5	3.0	4.0	3.0		3.8	3.1	3.9	0.0	4.1	5.6	2.8
	Pentacosane	13.6	38.1	72.3	36.1	36.6	29.1	15.5	2.7	24.6	3.2	2.3	0.0	5.9	4.8	0.0	2.3	0.0		0.0	0.0	0.0	0.0	0.0	0.0	0.0
	3 Me C25	3.1	2.9	5.6	3.8	4.0	3.7	2.2	0.8	2.2	1.9	1.8	0.8	3.2	1.6	0.6	3.9	1.5		3.7	4.1	3.0	1.7	5.2	5.1	3.1
	Heptacosene	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0	5.0	3.2	3.5	3.7	2.5	4.4	1.8		3.1	10.5	2.5	4.9	3.9	2.5	4.6
	Heptacosane	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	59.5			0.0	0.0	0.0	0.0	0.0	0.0
Percentage of M+3	Tricosene	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.8		0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Tricosane	14.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0	0.0
	3 Me C23	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6		0.0	0.0	0.0	5.8	0.0	0.0	0.0
	Pentacosene	1.1	0.0	1.2	0.3	0.2	0.4	0.4	0.0	0.0	0.8	0.2	0.3	0.2	1.0	0.0	0.1	0.1		0.0	0.0	0.2	0.0	0.4	0.0	0.3
	Pentacosane	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.9	0.0		0.0	0.0	0.0	0.0	0.0	0.0	0.0
	3 Me C25	1.0	0.7	0.8	0.0	0.6	1.9	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.7	0.6	0.8		1.9	0.0	0.0	1.4	0.0	0.0	0.0
	Heptacosene	0.9	0.2	0.0	0.0	0.4	1.0	0.6	0.0	0.0	0.6	0.0	0.0	0.0	0.0	0.9	0.0	0.0		0.0	0.0	1.3	1.3	0.0	0.0	0.8
	Heptacosane	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0			0.0	0.0	0.0	0.0	0.0	0.0

0
0-20
20-40
40-60
60-80
80-100
100+

Figure C.2: Data sheet showing page two of the raw control data for the L-leucine 5,5,5-d_3 experiment and *Formica lemani*.

L-Leucine 5,5,5-d_3		*Formica lemani*																								
		Leu 1	Leu 2	Leu 3	Leu 4	Leu 5	Leu 6	Leu 7	Leu 8	Leu 9	Leu 10	Leu 11	Leu 12	Leu 13	Leu 14	Leu 15	Leu 16	Leu 17	Leu 18	Leu 19	Leu 20	Leu 21	Leu 22	Leu 23	Leu 24	Leu 25
Abundance of M	Tricosene	3199	5600	7029	16648	8278	19016	19312	4376	4680	4591	6572	2448	3784	5659	22200	6177	4999	5218	14516	10050	13649	6460	9082	5278	8748
	Tricosane	3121	1475	2062	3511	1949	3182	2899	2800	840	1332	7829	1451	3605	2069	8084	3166	1188	4925	9332	6272	7597	2114	4114	2194	3372
	3 Me C23	6023	11658	14231	20480	9438	23872	25232	9954	5856	11342	7193	2738	6259	7004	33640	15136	10174	4487	12539	17944	25984	8211	11873	9784	12715
	Pentacosene	96424	152832	176576	334592	136448	410048	425344	105208	90704	143744	127488	41648	85552	117248	511296	227392	143232	127088	275264	277056	416960	145088	186112	138944	183040
	Pentacosane	3916	6924	2179	7609	6330	11369	11943	12890	5386	2433	12343	2860	5839	6377	8148	6248	5231	7756	14235	7117	23792	13059	7803	7679	3601
	3 Me C25	27816	33120	42600	65592	29168	90424	94064	40560	28208	31264	36512	12104	20880	30600	86352	49448	28688	33128	77552	58672	87016	33184	48088	30736	45488
	Heptacosene	37536	44496	43096	86064	26224	89872	100952	25032	23688	44440	48776	14385	20776	32512	116272	59360	34344	40776	71072	64368	102200	44328	42768	40120	41176
	Heptacosane	489	3393	1549	1088		2038	854	4567	1557	428	2524	935	1207		1375	997	163	2291	2358	2192	7635	1894	2897	1798	1471
Abundance of M+1	Tricosene	1530	1938	3108	4618	1094	7112	3335	1002	554	2495	1788	717	552	1083	5714	2352	1470	2672	3182	4000	8039	2434	1356	1603	1519
	Tricosane	2293	443	1393	1606		1301		359	1309		1521	310	1255		930	696	299	1666	3317	3060	1933	692	1937	848	985
	3 Me C23	2621	620	4851	6160	2038	4748	7701	3580	572	3495	1166		651	2075	8679	3270	1837	3351	6425	3324	3934	3117	4150	605	3822
	Pentacosene	27064	37816	41024	83776	32520	106976	97904	26112	23328	32008	32360	7698	20104	37552	140544	66208	37456	29008	70416	78264	119496	36624	35136	33888	45680
	Pentacosane	1353	2968	2135	2707		4328	2359	4716	874	215	1698	1528	3083	226	2575	821	3400	2888	3646	2953	5409	5033	1936	3090	1736
	3 Me C25	6106	12636	7626	11091	7014	24376	20192	7218	5886	9789	10874	2517	8813	8322	27184	15472	6193	8022	16656	16097	20952	14751	7989	8879	10249
	Heptacosene	3949	9448	12273	18464	13043	28864	28968	8971	7488	11501	9382	2962	6753	8005	33152	13742	11794	7458	20376	16688	32736	11870	12117	10811	16496
	Heptacosane		1557				1939		796	286		1018		365		665		277	492	1246	474	423	752	913		1315
Abundance of M+2	Tricosene	420				181	1304	3352								1050				353	498	824		1179		
	Tricosane																		202	1699	886					
	3 Me C23		722	921		372	432	435		439	7577					1565	246	989							718	
	Pentacosene	1081	5218	7150	10412	7228	13419	12053	5323	3741		4927	2288	4776	4828	21960	7503	4311	5018	5679	9668	11498	5963	9262	5964	3265
	Pentacosane						778		1087						174				384	473		274			344	
	3 Me C25	1751	269	942	4958	1238	3306	1852	365	1326	1240	2333		2478	569	3575	1815		717	1487	2075	4476	592	1116	1234	1058
	Heptacosene	3046	1497	1099	5500	1958	3375	2100	222	615	2076	2669	1588	1213	331	3250	4419	2132	873	1845	1324	4321	3121	1783	680	
	Heptacosane		775															499								
Abundance of M+3	Tricosene																									
	Tricosane																									
	3 Me C23																									
	Pentacosene		591	457	1762	842	1770	1872				357		328	827	1661	338	341	299	720	2304	799			757	
	Pentacosane																		554	838						
	3 Me C25	444		394															1002		552	189	1092			607
	Heptacosene		1008		344		888	581								717			394	538				227		
	Heptacosane																									

Figure C.3: Data sheet showing page one of the raw substrate data for the L-leucine 5,5,5-d_3 experiment and *Formica lemani*.

L-Leucine 5,5,5-d_3		*Formica lemani*																								
		Leu 1	Leu 2	Leu 3	Leu 4	Leu 5	Leu 6	Leu 7	Leu 8	Leu 9	Leu 10	Leu 11	Leu 12	Leu 13	Leu 14	Leu 15	Leu 16	Leu 17	Leu 18	Leu 19	Leu 20	Leu 21	Leu 22	Leu 23	Leu 24	Leu 25
Percentage of M+1	Tricosene	47.8	34.6	44.2	27.7	13.2	37.4	17.3	22.9	11.8	54.3	27.2	29.3	14.6	19.1	25.7	38.1	29.4	51.2	21.9	39.8	58.9	37.7	14.9	30.4	17.4
	Tricosane	73.5	30.0	67.6	45.7	0.0	40.9	0.0	12.8	155.8	0.0	19.4	21.4	34.8	0.0	11.5	22.0	25.2	33.8	35.5	48.8	25.4	32.7	47.1	38.7	29.2
	3 Me C23	43.5	5.3	34.1	30.1	21.6	19.9	30.5	36.0	9.8	30.8	16.2	0.0	10.4	29.6	25.8	21.6	18.1	74.7	51.2	18.5	15.1	38.0	35.0	6.2	30.1
	Pentacosene	28.1	24.7	23.2	25.0	23.8	26.1	23.0	24.8	25.7	22.3	25.4	18.5	23.5	32.0	27.5	29.1	26.2	22.8	25.6	28.2	28.7	25.2	18.9	24.4	25.0
	Pentacosane	34.6	42.9	98.0	35.6	0.0	38.1	19.8	36.6	16.2	8.8	13.8	53.4	52.8	3.5	31.6	13.1	65.0	37.2	25.6	41.5	22.7	38.5	24.8	40.2	48.2
	3 Me C25	22.0	38.2	17.9	16.9	24.0	27.0	21.5	17.8	20.9	31.3	29.8	20.8	42.2	27.2	31.5	31.3	21.6	24.2	21.5	27.4	24.1	44.5	16.6	28.9	22.5
	Heptacosene	10.5	21.2	28.5	21.5	49.7	32.1	28.7	35.8	31.6	25.9	19.2	20.6	32.5	24.6	28.5	23.2	34.3	18.3	28.7	25.9	32.0	26.8	28.3	26.9	40.1
	Heptacosane	0.0	45.9	0.0	0.0		95.1	0.0	17.4	18.4	0.0	40.3	0.0	30.2		48.4	0.0	169.9	21.5	52.8	21.6	5.5	39.7	31.5	0.0	89.4
Percentage of M+2	Tricosene	13.1	0.0	0.0	0.0	2.2	6.9	17.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.7	0.0	0.0	0.0	2.4	5.0	6.0	0.0	13.0	0.0	0.0
	Tricosane	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.1	18.2	14.1	0.0	0.0	0.0	0.0	0.0
	3 Me C23	0.0	6.2	6.5	0.0	3.9	1.8	1.7	0.0	7.5	66.8	0.0	0.0	0.0	0.0	4.7	1.6	9.7	0.0	0.0	0.0	0.0	0.0	0.0	7.3	0.0
	Pentacosene	1.1	3.4	4.0	3.1	5.3	3.3	2.8	5.1	4.1	0.0	3.9	5.5	5.6	4.1	4.3	3.3	3.0	3.9	2.1	3.5	2.8	4.1	5.0	4.3	1.8
	Pentacosane	0.0	0.0	0.0	0.0	0.0	6.8	0.0	8.4	0.0	0.0	0.0	0.0	0.0	2.7	0.0	0.0	0.0	5.0	3.3	0.0	1.2	0.0	0.0	4.5	0.0
	3 Me C25	6.3	0.8	2.2	7.6	4.2	3.7	2.0	0.9	4.7	4.0	6.4	0.0	11.9	1.9	4.1	3.7	0.0	2.2	1.9	3.5	5.1	1.8	2.3	4.0	2.3
	Heptacosene	8.1	3.4	2.6	6.4	7.5	3.8	2.1	0.9	2.6	4.7	5.5	11.0	5.8	1.0	2.8	7.4	6.2	2.1	2.6	2.1	4.2	7.0	4.2	1.7	0.0
	Heptacosane	0.0	22.8	0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.0	0.0	306.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Percentage of M+3	Tricosene	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Tricosane	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	3 Me C23	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Pentacosene	0.0	0.4	0.3	0.5	0.6	0.4	0.4	0.0	0.0	0.0	0.3	0.0	0.4	0.7	0.3	0.1	0.2	0.2	0.3	0.8	0.2	0.0	0.0	0.5	0.0
	Pentacosane	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.1	5.9	0.0	0.0	0.0	0.0	0.0	0.0
	3 Me C25	1.6	0.0	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.0	0.0	0.9	0.2	3.3	0.0	0.0	1.3
	Heptacosene	0.0	2.3	0.0	0.4	0.0	1.0	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.0	0.0	1.0	0.8	0.0	0.0	0.0	0.5	0.0	0.0
	Heptacosane	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

0
0-20
20-40
40-60
60-80
80-100
100+

Figure C.4: Data sheet showing page two of the raw substrate data for the L-leucine 5,5,5-d_3 experiment and *Formica lemani*.

L-Leucine (Negative Control)		*Myrmica sabuleti*						
		Con 1	Con 2	Con 3	Con 4	Con 5	Con 6	Con 7
Abundance of M	Tricosane	10535	4459	12414	10962	4105	9145	10148
	5 Me C23	8365	3788	14595	21480	10722	8942	9624
	Pentacosene	107520	35112	92288	84280	92008	88856	60720
	Pentacosane	4407	1287	4864	4965	5398	3493	4400
	11 13 Me C25	29192	11404	31264	34712	25728	23800	17456
	5 Me C25	60728	14194	59264	54776	42528	57592	30768
	Heptacosene	16123	6783	22432	30880	15868	24832	8606
	5 Me C27	17216	4438	21248	21032	13638	19976	8682
Abundance of M+1	Tricosane	1311	936	2503	4230	2375	3915	2884
	5 Me C23	3302	1259	4096	3705	1695	3786	466
	Pentacosene	24040	7690	25832	22080	24168	23712	18192
	Pentacosane	1759			1073	1488		3317
	11 13 Me C25	13831	7103	20240	10863	12630	16182	10242
	5 Me C25	11859	4171	10868	10252	8526	10623	6370
	Heptacosene	6192	2237	6490	6351	5899	8603	4603
	5 Me C27	3453	2504	4390	3734	2777	4749	1302
Abundance of M+2	Tricosane	358			956			
	5 Me C23	397			440	362	718	
	Pentacosene	3961	1648	5816	1881	3863	6161	3835
	Pentacosane	276						556
	11 13 Me C25	705	1027		870	957	1709	1862
	5 Me C25	3312	301	1472	1218	249	1598	1341
	Heptacosene	231		759	1927	636	812	1209
	5 Me C27	1912	177	332		487	239	
Abundance of M+3	Tricosane							
	5 Me C23							
	Pentacosene				260			
	Pentacosane							
	11 13 Me C25							
	5 Me C25				835			
	Heptacosene	296						
	5 Me C27					1015		

Figure C.5: Data sheet showing page one of the raw control data for the L-leucine 5,5,5-d_3 experiment and *Myrmica sabuleti*.

L-Leucine (Negative Control)		*Myrmica sabuleti*						
		Con 1	Con 2	Con 3	Con 4	Con 5	Con 6	Con 7
Percentage of M+1	Tricosane	12.4	21.0	20.2	38.6	57.9	42.8	28.4
	5 Me C23	39.5	33.2	28.1	17.2	15.8	42.3	4.8
	Pentacosene	22.4	21.9	28.0	26.2	26.3	26.7	30.0
	Pentacosane	39.9	0.0	0.0	21.6	27.6	0.0	75.4
	11 13 Me C25	47.4	62.3	64.7	31.3	49.1	68.0	58.7
	5 Me C25	19.5	29.4	18.3	18.7	20.0	18.4	20.7
	Heptacosene	38.4	33.0	28.9	20.6	37.2	34.6	53.5
	5 Me C27	20.1	56.4	20.7	17.8	20.4	23.8	15.0
Percentage of M+2	Tricosane	3.4	0.0	0.0	8.7	0.0	0.0	0.0
	5 Me C23	4.7	0.0	0.0	2.0	3.4	8.0	0.0
	Pentacosene	3.7	4.7	6.3	2.2	4.2	6.9	6.3
	Pentacosane	6.3	0.0	0.0	0.0	0.0	0.0	12.6
	11 13 Me C25	2.4	9.0	0.0	2.5	3.7	7.2	10.7
	5 Me C25	5.5	2.1	2.5	2.2	0.6	2.8	4.4
	Heptacosene	1.4	0.0	3.4	6.2	4.0	3.3	14.0
	5 Me C27	11.1	4.0	1.6	0.0	3.6	1.2	0.0
Percentage of M+3	Tricosane	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	5 Me C23	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Pentacosene	0.0	0.0	0.0	0.3	0.0	0.0	0.0
	Pentacosane	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	11 13 Me C25	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	5 Me C25	0.0	0.0	0.0	1.5	0.0	0.0	0.0
	Heptacosene	1.8	0.0	0.0	0.0	0.0	0.0	0.0
	5 Me C27	0.0	0.0	0.0	0.0	7.4	0.0	0.0

0
0-20
20-40
40-60
60-80
80-100
100+

Figure C.6: Data sheet showing page two of the raw control data for the L-leucine 5,5,5-d_3 experiment and *Myrmica sabuleti*.

L-Leucine 5,5,5-d_3		*Myrmica sabuleti*																		
		Leu 1	Leu 2	Leu 3	Leu 4	Leu 5	Leu 6	Leu 7	Leu 8	Leu 9	Leu 10	Leu 11	Leu 12	Leu 13	Leu 14	Leu 15	Leu 16	Leu 17	Leu 18	Leu 19
Abundance of M	Tricosane	7117	2930	7424	2318	18624	4064	3637	5811	10829	5022	6190	2777	12211		5695	4818	2694	6066	7185
	5 Me C23	12010	1069	4029	8100	19120	6722	3445	8112	8278	6838	5354	2880	22552		9761	4873	6435	10411	5456
	Pentacosene	96624	19936	60920	53128	158656	51752	53744	73552	76496	74144	71600	46248	159424		102024	63912	44432	80600	50016
	Pentacosane	2225	3821	1695	3141	5586	1499	1241	1614	1863	2094	4745	759	4726		4207	3851	2160	1023	4365
	11 13 Me C25	28520	4234	14652	9854	25976	14959	5312	11165	13991	12917	7142	7896	46408		15092	10563	10311	19296	11639
	5 Me C25	50464	6147	29096	20080	85152	40032	24016	43840	40816	36568	39560	21672	96416		38896	36368	15476	55256	25056
	Heptacosene	17080	4820	8838	12310	24936	10457	7289	15303	17344	12872	10428	10771	35008		17328	15146	7860	12312	10976
	5 Me C27	10904	2518	9018	5253	17200	8296		6202	7920	7578	10614	3671	25816		10206	102752	3762	14284	10308
Abundance of M+1	Tricosane	4357	1250	2995	3486	5331	1183	1117	1748	2712	1045	1876	204	4535		3067	1992	1996	1013	1325
	5 Me C23	3747		489	1072	8624	1349	1705	1834	2383	2188	1704	1601	4126		1896	836	1147	1126	
	Pentacosene	28592	5565	23416	25000	49152	15017	22048	21920	19096	25640	27680	16152	49272		29048	22264	13168	32448	22152
	Pentacosane	1281	163	785	1536	1647	341		1525	675	1353	1757		3144		1042	758	684	1414	566
	11 13 Me C25	13087	3764	3465	7069	16086	7194	5790	7607	11238	9190	5081	2815	20744		7556	9734	7341	16472	4224
	5 Me C25	13416	3438	4785	5266	11299	9747	5346	8858	10969	8620	8038	5727	20368		15541	6814	4111	9885	3333
	Heptacosene	8825	766	4623	3530	10830	4086	3941	3189	6673	2999	5052	3998	13836		3951	5591	6523	5183	4078
	5 Me C27	4212		1678	4424	7392	2486		1518	7255	1640	2749	206	6423		4094	3364	1297	3085	2549
Abundance of M+2	Tricosane	344		1164	178	1218		610		642	736	495	293						235	384
	5 Me C23							1224			553	625				469		939		
	Pentacosene	6991	1960	670	4336	4606	2354	3311	4045	9219	3664	5819	1766	7140		3800	4524	2800	6530	4041
	Pentacosane			284	744	151								583		1054				
	11 13 Me C25	2321	974		884	3933	2499	1492	326	1594	317	836	333	3924					2419	1520
	5 Me C25	2677	236	585	303	2510	1397	405	639	2314	951	1343		1636		1793	481	3461	1138	920
	Heptacosene	1242			1345	3438		523	2525	954	1773	1742	768	3188		3176		1229	1306	
	5 Me C27	2172					542		257	166	1247	1300		454		1175	283	366		
Abundance of M+3	Tricosane																			
	5 Me C23																			
	Pentacosene	1015			237					254	150			1471		1438	211			
	Pentacosane				202															
	11 13 Me C25	698			163			790												
	5 Me C25	213												689						
	Heptacosene					423						469								
	5 Me C27											640					398			

Figure C.7: Data sheet showing page one of the raw substrate data for the L-leucine 5,5,5-d_3 experiment and *Myrmica sabuleti*.

L-Leucine 5,5,5-d_3		*Myrmica sabuleti*																		
		Leu 1	Leu 2	Leu 3	Leu 4	Leu 5	Leu 6	Leu 7	Leu 8	Leu 9	Leu 10	Leu 11	Leu 12	Leu 13	Leu 14	Leu 15	Leu 16	Leu 17	Leu 18	Leu 19
Percentage of M+1	Tricosane	61.2	42.7	40.3	150.4	28.6	29.1	30.7	30.1	25.0	20.8	30.3	7.3	37.1		53.9	41.3	74.1	16.7	18.4
	5 Me C23	31.2	0.0	12.1	13.2	45.1	20.1	49.5	22.6	28.8	32.0	31.8	55.6	18.3		19.4	17.2	17.8	10.8	0.0
	Pentacosene	29.6	27.9	38.4	47.1	31.0	29.0	41.0	29.8	25.0	34.6	38.7	34.9	30.9		28.5	34.8	29.6	40.3	44.3
	Pentacosane	57.6	4.3	46.3	48.9	29.5	22.7	0.0	94.5	36.2	64.6	37.0	0.0	66.5		24.8	19.7	31.7	138.2	13.0
	11 13 Me C25	45.9	88.9	23.6	71.7	61.9	48.1	109.0	68.1	80.3	71.1	71.1	35.7	44.7		50.1	92.2	71.2	85.4	36.3
	5 Me C25	26.6	55.9	16.4	26.2	13.3	24.3	22.3	20.2	26.9	23.6	20.3	26.4	21.1		40.0	18.7	26.6	17.9	13.3
	Heptacosene	51.7	15.9	52.3	28.7	43.4	39.1	54.1	20.8	38.5	23.3	48.4	37.1	39.5		22.8	36.9	83.0	42.1	37.2
	5 Me C27	38.6	0.0	18.6	84.2	43.0	30.0		24.5	91.6	21.6	25.9	5.6	24.9		40.1	3.3	34.5	21.6	24.7
Percentage of M+2	Tricosane	4.8	0.0	15.7	7.7	6.5	0.0	16.8	0.0	5.9	14.7	8.0	10.6	0.0		0.0	0.0	0.0	3.9	5.3
	5 Me C23	0.0	0.0	0.0	0.0	0.0	0.0	35.5	0.0	0.0	8.1	11.7	0.0	0.0		4.8	0.0	14.6	0.0	0.0
	Pentacosene	7.2	9.8	1.1	8.2	2.9	4.5	6.2	5.5	12.1	4.9	8.1	3.8	4.5		3.7	7.1	6.3	8.1	8.1
	Pentacosane	0.0	0.0	16.8	23.7	2.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	12.3		25.1	0.0	0.0	0.0	0.0
	11 13 Me C25	8.1	23.0	0.0	9.0	15.1	16.7	28.1	2.9	11.4	2.5	11.7	4.2	8.5		0.0	0.0	0.0	12.5	13.1
	5 Me C25	5.3	3.8	2.0	1.5	2.9	3.5	1.7	1.5	5.7	2.6	3.4	0.0	1.7		4.6	1.3	22.4	2.1	3.7
	Heptacosene	7.3	0.0	0.0	10.9	13.8	0.0	7.2	16.5	5.5	13.8	16.7	7.1	9.1		18.3	0.0	15.6	10.6	0.0
	5 Me C27	19.9	0.0	0.0	0.0	0.0	6.5		4.1	2.1	16.5	12.2	0.0	1.8		11.5	0.3	9.7	0.0	0.0
Percentage of M+3	Tricosane	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0
	5 Me C23	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0
	Pentacosene	1.1	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.3	0.2	0.0	0.0	0.9		1.4	0.3	0.0	0.0	0.0
	Pentacosane	0.0	0.0	0.0	6.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0
	11 13 Me C25	2.4	0.0	0.0	1.7	0.0	0.0	14.9	0.0	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0
	5 Me C25	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7		0.0	0.0	0.0	0.0	0.0
	Heptacosene	0.0	0.0	0.0	0.0	1.7	0.0	0.0	0.0	0.0	0.0	4.5	0.0	0.0		0.0	0.0	0.0	0.0	0.0
	5 Me C27	0.0	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	6.0	0.0	0.0		0.0	0.4	0.0	0.0	0.0

0
0-20
20-40
40-60
60-80
80-100
100+

Figure C.8: Data sheet showing page two of the raw substrateL-leucine 5,5,5-d_3 experiment and *Myrmica sabuleti*.

L-Leucine (Negative Control)		*Formica lemani*								
		Con 1	Con 2	Con 3	Con 4	Con 5	Con 6	Con 7	Con 8	Con 9
Abundance of M	3 Me C23		1149	3050	1436	7418	2260	11946	4036	3021
	Pentacosene		51200	56600	54736	77392	62776	81720	76888	56624
	Pentacosane		2028	12016	8329	6022	2858	9848	18528	12888
	3 Me C25		7403	11945	8723	25768	11723	37888	22560	11500
	Heptacosene		27472	29088	30896	46864	31728	55376	45992	29488
	Heptacosane		247	4388	3645	1704	680	2564	7034	5243
	3 Me C27		2069	6077	5657	7647	3711	122230	10013	8639
	Nonacosene		1061	1923	1715	5077	1557	7040	3921	2185
Abundance of M+1	3 Me C23		234	657	374	1545	586	2568	1040	815
	Pentacosene		14033	14930	14149	19864	16024	20712	20360	15358
	Pentacosane		551	3184	2243	1705	881	2714	4999	3187
	3 Me C25		1880	3113	2034	6551	2999	9831	5637	3082
	Heptacosene		8017	8245	9253	13925	9731	16672	13485	8440
	Heptacosane			1317	1028	531	182	739	1989	1557
	3 Me C27		581	1679	1585	2100	999	3174	3022	2403
	Nonacosene		350	582	458	1682	560	2372	1217	818
Abundance of M+2	3 Me C23					176		332		
	Pentacosene		1843	1979	1959	2698	2202	2728	2458	1986
	Pentacosane			466	345	213		362	699	538
	3 Me C25		217	393	263	807	371	1323	711	394
	Heptacosene		1052	1214	1259	1991	1364	2100	1827	1214
	Heptacosane			182	186				302	204
	3 Me C27			216	211	291	150	480	437	284
	Nonacosene					252		333	166	
Abundance of M+3	3 Me C23									
	Pentacosene									
	Pentacosane									
	3 Me C25									
	Heptacosene									
	Heptacosane									
	3 Me C27									
	Nonacosene									

Figure C.9: Data sheet showing page one of the raw control data for the L-leucine $^{13}C_6$,^{15}N experiment and *Formica lemani*.

(Negative Control)		Con 1	Con 2	Con 3	Con 4	Con 5	Con 6	Con 7	Con 8	Con 9
Percentage of M+1	3 Me C23		20.4	21.5	26.0	20.8	25.9	21.5	25.8	27.0
	Pentacosene		27.4	26.4	25.8	25.7	25.5	25.3	26.5	27.1
	Pentacosane		27.2	26.5	26.9	28.3	30.8	27.6	27.0	24.7
	3 Me C25		25.4	26.1	23.3	25.4	25.6	25.9	25.0	26.8
	Heptacosene		29.2	28.3	29.9	29.7	30.7	30.1	29.3	28.6
	Heptacosane		0.0	30.0	28.2	31.2	26.8	28.8	28.3	29.7
	3 Me C27		28.1	27.6	28.0	27.5	26.9	2.6	30.2	27.8
	Nonacosene		33.0	30.3	26.7	33.1	36.0	33.7	31.0	37.4
Percentage of M+2	3 Me C23		0.0	0.0	0.0	2.4	0.0	2.8	0.0	0.0
	Pentacosene		3.6	3.5	3.6	3.5	3.5	3.3	3.2	3.5
	Pentacosane		0.0	3.9	4.1	3.5	0.0	3.7	3.8	4.2
	3 Me C25		2.9	3.3	3.0	3.1	3.2	3.5	3.2	3.4
	Heptacosene		3.8	4.2	4.1	4.2	4.3	3.8	4.0	4.1
	Heptacosane		0.0	4.1	5.1	0.0	0.0	0.0	4.3	3.9
	3 Me C27		0.0	3.6	3.7	3.8	4.0	0.4	4.4	3.3
	Nonacosene		0.0	0.0	0.0	5.0	0.0	4.7	4.2	0.0
Percentage of M+3	3 Me C23		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Pentacosene		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Pentacosane		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	3 Me C25		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Heptacosene		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Heptacosane		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	3 Me C27		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Nonacosene		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

0
0-20
20-40
40-60
60-80
80-100
100+

Figure C.10: Data sheet showing page two of the raw control data for the L-leucine $^{13}C_6$,^{15}N experiment and *Formica lemani*.

L-Leucine $^{13}C_6$, ^{15}N		*Formica lemani*													
		Leu 1	Leu 2	Leu 3	Leu 4	Leu 5	Leu 6	Leu 7	Leu 8	Leu 9	Leu 10	Leu 11	Leu 12	Leu 13	Leu 14
Abundance of M	3 Me C23	625	2456	1234	4286	1866	1845	2372	481	2236	891	450	2481	379	3813
	Pentacosene	15929	20880	17480	50808	23056	20928	43640	14031	21704	16253	11921	17352	16279	25624
	Pentacosane	3045	3839	1970	3912	2353	2152	3809	728	2234	2333	926	4140	1523	3279
	3 Me C25	3762	11281	5126	21128	9532	8334	11034	2404	12259	4751	2179	12598	5941	17544
	Heptacosene	7136	12039	9192	27512	11926	10863	20888	5735	13160	7549	4924	10521	8352	16464
	Heptacosane	1629	1610	631	1108	501	574	1094	158	322	978	288	2014	1523	821
	3 Me C27	3214	4117	2070	6347	2553	2597	3991	711	4024	1841	774	6336	1984	4582
	Nonacosene	283	1487	595	2702	866	1006	1297	198	1031	307	158	1506	599	1689
Abundance of M+1	3 Me C23	216	812	351	1300	517	566	757	209	742	293	150	772	151	1077
	Pentacosene	5919	7535	6689	19344	8466	7729	16504	5036	7749	5982	4733	6348	5894	9777
	Pentacosane	1153	1521	734	1519	780	779	1214	269	739	818	412	1504	554	1141
	3 Me C25	1236	3632	1831	6516	2824	2464	3631	792	3674	1414	747	3927	1920	5291
	Heptacosene	3015	5093	3890	12876	5246	4577	9086	2379	5636	3413	2310	4686	3307	7007
	Heptacosane	542	571	231	472	176	251	474			395		734	554	338
	3 Me C27	1025	1515	698	2218	777	779	1397	251	1165	605	263	2044	597	1563
	Nonacosene	194	761	351	1584	426	501	720		540	197		798	272	878
Abundance of M+2	3 Me C23		447	207	706	287	310	480		440	157		396		636
	Pentacosene	3108	4006	3370	10306	4328	3977	9271	2819	4125	3414	2424	3349	2903	5013
	Pentacosane	596	896	513	1079	354	459	791		356	478	222	814	297	602
	3 Me C25	571	1533	752	2785	1024	1129	1438	354	1397	618	289	1572	710	2274
	Heptacosene	1989	3496	2532	8432	3528	3232	6318	1606	3421	2271	1519	3051	2233	4598
	Heptacosane	215	384	179	345			170			254		297	297	
	3 Me C27	540	845	310	1078	311	397	516	352	477	310	152	839	277	639
	Nonacosene		600	237	1264	368	437	678		454	198		665	228	694
Abundance of M+3	3 Me C23		455	181	745	237	292	374		380			381		584
	Pentacosene	2043	2600	2285	6860	2955	2775	6233	1903	2597	2250	1745	2268	1924	3453
	Pentacosane	466	818	478	957	305	440	666		257	439	222	618	227	481
	3 Me C25	540	1525	763	2693	1008	981	1434	260	1445	580	266	1452	657	2010
	Heptacosene	1429	2637	1790	6407	2599	2353	4828	1320	2602	1720	1196	2239	1676	3410
	Heptacosane	200	337	183	356			163			169		288	227	
	3 Me C27	493	784	285	1126	277	399	458		384	316		818	259	728
	Nonacosene		563	238	1166	267	354	540		375			543	203	696
Abundance of M+4	3 Me C23		484	204	757	333	277	444		493			422		700
	Pentacosene	1536	2071	1627	5183	1396	2030	4812	1406	1963	1797	1191	1743	1366	2583
	Pentacosane	482	858	490	1049	272	380	670		243	430	201	632	200	493
	3 Me C25	453	1584	852	2780	1108	974	1549	309	1565	651	310	1599	827	2373
	Heptacosene	1171	2159	1613	5355	2288	2084	4353	1079	2135	1454	984	1910	1398	2963
	Heptacosane	190	349	182	305			162			223		303	200	
	3 Me C27	507	844	383	1112	308	424	527	193	499	356		912	272	756
	Nonacosene		460	220	1113	274	333	507		326			593	171	620

Figure C.11: Data sheet showing page one of the raw substrate data for the L-leucine $^{13}C_6$,^{15}N experiment and *Formica lemani*.

L-Leucine $^{13}C_6$, ^{15}N		*Formica lemani*													
		Leu 1	Leu 2	Leu 3	Leu 4	Leu 5	Leu 6	Leu 7	Leu 8	Leu 9	Leu 10	Leu 11	Leu 12	Leu 13	Leu 14
Abundance of M+5	3 Me C23		375	160	702	295	293	370		455			374		674
	Pentacosene	982	1410	1207	3811	1036	1471	3619	951	1364	1225	852	1340	984	1677
	Pentacosane	354	697	438	1080	280	376	590		218	426	173	645	168	411
	3 Me C25	421	1439	688	2864	982	1024	1387	319	1577	566	282	1489	817	2307
	Heptacosene	841	1534	1151	4105	1508	1540	3196	806	1558	1140	679	1507	934	2012
	Heptacosane	180	317	157	437			183			216		271	168	
	3 Me C27	439	812	332	1150	325	474	578		486	345		945	335	828
	Nonacosene		401	186	768	234	276	422		327			507		504
Abundance of M+6	3 Me C23		316		656	290	217	454		395			357		585
	Pentacosene	759	1057	939	3016	742	1015	2752	685	929	1024	685	991	672	1279
	Pentacosane	317	610	453	1102	197	343	616		208	437		633	172	355
	3 Me C25	372	1325	709	2633	1036	1003	1451	288	1582	556	253	1442	797	2210
	Heptacosene	655	1228	889	2987	1102	1094	2485	607	1145	880	523	1117	719	1495
	Heptacosane		323		337			188			251		279	172	
	3 Me C27	398	762	354	1274	359	451	590		486	329		916	342	787
	Nonacosene		356		632	160	213	375		201			391		363
Abundance of M+7	3 Me C23		261		450	207	206	316		335			302		523
	Pentacosene	554	823	699	2267	551	883	2219	509	690	777	558	714	513	948
	Pentacosane	272	536	369	876	198	290	489		157	366		539		320
	3 Me C25	287	1007	573	2172	951	759	1082	321	1237	409	257	1145	681	1810
	Heptacosene	462	877	659	2330	764	828	1866	376	777	745	451	881	455	1187
	Heptacosane		268		382						192		310		
	3 Me C27	298	611	282	1153	281	437	505		511	272		774	275	774
	Nonacosene		224		447		157	247		175			324		327
Abundance of M+8	3 Me C23		222		354		155	220		289			254		426
	Pentacosene	425	698	578	1814	405	650	1601	427	588	641	432	636	374	813
	Pentacosane	257	378	338	856		264	482		150	299		449		300
	3 Me C25	219	920	422	1883	788	622	834	247	1057	373	175	839	544	1495
	Heptacosene	356	735	499	1836	591	626	1544	297	540	526	340	699	348	811
	Heptacosane		211		287								274		
	3 Me C27	198	489	219	899	293	354	425		380	212		647	300	705
	Nonacosene		196		341			312					248		208
Abundance of M+9	3 Me C23				248			163		194			184		291
	Pentacosene	361	511	439	1442	340	557	1320	349	416	475	412	449	282	633
	Pentacosane	169	346	258	732		203	344			213		389		253
	3 Me C25		643	352	1326	568	460	596	194	972	230	154	646	479	1131
	Heptacosene	299	556	420	1530	424	492	1208	195	420	428	265	513	264	655
	Heptacosane		163		315								212		
	3 Me C27	171	323	169	765	248	287	305		399	178		414	229	544
	Nonacosene		189		335			178					233		203
Abundance of M+10	3 Me C23				200										191
	Pentacosene	254	336	333	1019	248	357	964	284	320	346	268	364	232	444
	Pentacosane		181	186	561			213			157		262		166
	3 Me C25		366	239	928	442	382	510		648	157		463	337	906
	Heptacosene	228	392	307	1109	303	341	883	178	312	304	174	385	190	524
	Heptacosane				240										
	3 Me C27		241		570	203	193	232		249			380	202	483
	Nonacosene				288			171					163		162

Figure C.12: Data sheet showing page two of the raw substrate data for the L-leucine $^{13}C_6$, ^{15}N experiment and *Formica lemani*.

L-Leucine $^{13}C_6$, ^{15}N		*Formica lemani*													
		Leu 1	Leu 2	Leu 3	Leu 4	Leu 5	Leu 6	Leu 7	Leu 8	Leu 9	Leu 10	Leu 11	Leu 12	Leu 13	Leu 14
Percentage of M+1	3 Me C23	34.6	33.1	28.4	30.3	27.7	30.7	31.9	43.5	33.2	32.9	33.3	31.1	39.8	28.2
	Pentacosene	37.2	36.1	38.3	38.1	36.7	36.9	37.8	35.9	35.7	36.8	39.7	36.6	36.2	38.2
	Pentacosane	37.9	39.6	37.3	38.8	33.1	36.2	31.9	37.0	33.1	35.1	44.5	36.3	36.4	34.8
	3 Me C25	32.9	32.2	35.7	30.8	29.6	29.6	32.9	32.9	30.0	29.8	34.3	31.2	32.3	30.2
	Heptacosene	42.3	42.3	42.3	46.8	44.0	42.1	43.5	41.5	42.8	45.2	46.9	44.5	39.6	42.6
	Heptacosane	33.3	35.5	36.6	42.6	35.1	43.7	43.3	0.0	0.0	40.4	0.0	36.4	36.4	41.2
	3 Me C27	31.9	36.8	33.7	34.9	30.4	30.0	35.0	35.3	29.0	32.9	34.0	32.3	30.1	34.1
	Nonacosene	68.6	51.2	59.0	58.6	49.2	49.8	55.5	0.0	52.4	64.2	0.0	53.0	45.4	52.0
Percentage of M+2	3 Me C23	0.0	18.2	16.8	16.5	15.4	16.8	20.2	0.0	19.7	17.6	0.0	16.0	0.0	16.7
	Pentacosene	19.5	19.2	19.3	20.3	18.8	19.0	21.2	20.1	19.0	21.0	20.3	19.3	17.8	19.6
	Pentacosane	19.6	23.3	26.0	27.6	15.0	21.3	20.8	0.0	15.9	20.5	24.0	19.7	19.5	18.4
	3 Me C25	15.2	13.6	14.7	13.2	10.7	13.5	13.0	14.7	11.4	13.0	13.3	12.5	12.0	13.0
	Heptacosene	27.9	29.0	27.5	30.6	29.6	29.8	30.2	28.0	26.0	30.1	30.8	29.0	26.7	27.9
	Heptacosane	13.2	23.9	28.4	31.1	0.0	0.0	15.5	0.0	0.0	26.0	0.0	14.7	19.5	0.0
	3 Me C27	16.8	20.5	15.0	17.0	12.2	15.3	12.9	49.5	11.9	16.8	19.6	13.2	14.0	13.9
	Nonacosene	0.0	40.3	39.8	46.8	42.5	43.4	52.3	0.0	44.0	64.5	0.0	44.2	38.1	41.1
Percentage of M+3	3 Me C23	0.0	18.5	14.7	17.4	12.7	15.8	15.8	0.0	17.0	0.0	0.0	15.4	0.0	15.3
	Pentacosene	12.8	12.5	13.1	13.5	12.8	13.3	14.3	13.6	12.0	13.8	14.6	13.1	11.8	13.5
	Pentacosane	15.3	21.3	24.3	24.5	13.0	20.4	17.5	0.0	11.5	18.8	24.0	14.9	14.9	14.7
	3 Me C25	14.4	13.5	14.9	12.7	10.6	11.8	13.0	10.8	11.8	12.2	12.2	11.5	11.1	11.5
	Heptacosene	20.0	21.9	19.5	23.3	21.8	21.7	23.1	23.0	19.8	22.8	24.3	21.3	20.1	20.7
	Heptacosane	12.3	20.9	29.0	32.1	0.0	0.0	14.9	0.0	0.0	17.3	0.0	14.3	14.9	0.0
	3 Me C27	15.3	19.0	13.8	17.7	10.8	15.4	11.5	0.0	9.5	17.2	0.0	12.9	13.1	15.9
	Nonacosene	0.0	37.9	40.0	43.2	30.8	35.2	41.6	0.0	36.4	0.0	0.0	36.1	33.9	41.2
Percentage of M+4	3 Me C23	0.0	19.7	16.5	17.7	17.8	15.0	18.7	0.0	22.0	0.0	0.0	17.0	0.0	18.4
	Pentacosene	9.6	9.9	9.3	10.2	6.1	9.7	11.0	10.0	9.0	11.1	10.0	10.0	8.4	10.1
	Pentacosane	15.8	22.3	24.9	26.8	11.6	17.7	17.6	0.0	10.9	18.4	21.7	15.3	13.1	15.0
	3 Me C25	12.0	14.0	16.6	13.2	11.6	11.7	14.0	12.9	12.8	13.7	14.2	12.7	13.9	13.5
	Heptacosene	16.4	17.9	17.5	19.5	19.2	19.2	20.8	18.8	16.2	19.3	20.0	18.2	16.7	18.0
	Heptacosane	11.7	21.7	28.8	27.5	0.0	0.0	14.8	0.0	0.0	22.8	0.0	15.0	13.1	0.0
	3 Me C27	15.8	20.5	18.5	17.5	12.1	16.3	13.2	27.1	12.4	19.3	0.0	14.4	13.7	16.5
	Nonacosene	0.0	30.9	37.0	41.2	31.6	33.1	39.1	0.0	31.6	0.0	0.0	39.4	28.5	36.7
Percentage of M+5	3 Me C23	0.0	15.3	13.0	16.4	15.8	15.9	15.6	0.0	20.3	0.0	0.0	15.1	0.0	17.7
	Pentacosene	6.2	6.8	6.9	7.5	4.5	7.0	8.3	6.8	6.3	7.5	7.1	7.7	6.0	6.5
	Pentacosane	11.6	18.2	22.2	27.6	11.9	17.5	15.5	0.0	9.8	18.3	18.7	15.6	11.0	12.5
	3 Me C25	11.2	12.8	13.4	13.6	10.3	12.3	12.6	13.3	12.9	11.9	12.9	11.8	13.8	13.1
	Heptacosene	11.8	12.7	12.5	14.9	12.6	14.2	15.3	14.1	11.8	15.1	13.8	14.3	11.2	12.2
	Heptacosane	11.0	19.7	24.9	39.4	0.0	0.0	16.7	0.0	0.0	22.1	0.0	13.5	11.0	0.0
	3 Me C27	13.7	19.7	16.0	18.1	12.7	18.3	14.5	0.0	12.1	18.7	0.0	14.9	16.9	18.1
	Nonacosene	0.0	27.0	31.3	28.4	27.0	27.4	32.5	0.0	31.7	0.0	0.0	33.7	0.0	29.8
Percentage of M+6	3 Me C23	0.0	12.9	0.0	15.3	15.5	11.8	19.1	0.0	17.7	0.0	0.0	14.4	0.0	15.3
	Pentacosene	4.8	5.1	5.4	5.9	3.2	4.8	6.3	4.9	4.3	6.3	5.7	5.7	4.1	5.0
	Pentacosane	10.4	15.9	23.0	28.2	8.4	15.9	16.2	0.0	9.3	18.7	0.0	15.3	11.3	10.8
	3 Me C25	9.9	11.7	13.8	12.5	10.9	12.0	13.2	12.0	12.9	11.7	11.6	11.4	13.4	12.6
	Heptacosene	9.2	10.2	9.7	10.9	9.2	10.1	11.9	10.6	8.7	11.7	10.6	10.6	8.6	9.1
	Heptacosane	0.0	20.1	0.0	30.4	0.0	0.0	17.2	0.0	0.0	25.7	0.0	13.9	11.3	0.0
	3 Me C27	12.4	18.5	17.1	20.1	14.1	17.4	14.8	0.0	12.1	17.9	0.0	14.5	17.2	17.2
	Nonacosene	0.0	23.9	0.0	23.4	18.5	21.2	28.9	0.0	19.5	0.0	0.0	26.0	0.0	21.5

0
0-20
20-40
40-60
60-80
80-100
100+

Figure C.13: Data sheet showing page three of the raw substrate data for the L-leucine $^{13}C_6$, ^{15}N experiment and *Formica lemani*.

L-Leucine $^{13}C_6$, ^{15}N		*Formica lemani*													
		Leu 1	Leu 2	Leu 3	Leu 4	Leu 5	Leu 6	Leu 7	Leu 8	Leu 9	Leu 10	Leu 11	Leu 12	Leu 13	Leu 14
Percentage of M+7	3 Me C23	0.0	10.6	0.0	10.5	11.1	11.2	13.3	0.0	15.0	0.0	0.0	12.2	0.0	13.7
	Pentacosene	3.5	3.9	4.0	4.5	2.4	4.2	5.1	3.6	3.2	4.8	4.7	4.1	3.2	3.7
	Pentacosane	8.9	14.0	18.7	22.4	8.4	13.5	12.8	0.0	7.0	15.7	0.0	13.0	0.0	9.8
	3 Me C25	7.6	8.9	11.2	10.3	10.0	9.1	9.8	13.4	10.1	8.6	11.8	9.1	11.5	10.3
	Heptacosene	6.5	7.3	7.2	8.5	6.4	7.6	8.9	6.6	5.9	9.9	9.2	8.4	5.4	7.2
	Heptacosane	0.0	16.6	0.0	34.5	0.0	0.0	0.0	0.0	0.0	19.6	0.0	15.4	0.0	0.0
	3 Me C27	9.3	14.8	13.6	18.2	11.0	16.8	12.7	0.0	12.7	14.8	0.0	12.2	13.9	16.9
	Nonacosene	0.0	15.1	0.0	16.5	0.0	15.6	19.0	0.0	17.0	0.0	0.0	21.5	0.0	19.4
Percentage of M+8	3 Me C23	0.0	9.0	0.0	8.3	0.0	8.4	9.3	0.0	12.9	0.0	0.0	10.2	0.0	11.2
	Pentacosene	2.7	3.3	3.3	3.6	1.8	3.1	3.7	3.0	2.7	3.9	3.6	3.7	2.3	3.2
	Pentacosane	8.4	9.8	17.2	21.9	0.0	12.3	12.7	0.0	6.7	12.8	0.0	10.8	0.0	9.1
	3 Me C25	5.8	8.2	8.2	8.9	8.3	7.5	7.6	10.3	8.6	7.9	8.0	6.7	9.2	8.5
	Heptacosene	5.0	6.1	5.4	6.7	5.0	5.8	7.4	5.2	4.1	7.0	6.9	6.6	4.2	4.9
	Heptacosane	0.0	13.1	0.0	25.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	13.6	0.0	0.0
	3 Me C27	6.2	11.9	10.6	14.2	11.5	13.6	10.6	0.0	9.4	11.5	0.0	10.2	15.1	15.4
	Nonacosene	0.0	13.2	0.0	12.6	0.0	0.0	24.1	0.0	0.0	0.0	0.0	16.5	0.0	12.3
Percentage of M+9	3 Me C23	0.0	0.0	0.0	5.8	0.0	0.0	6.9	0.0	8.7	0.0	0.0	7.4	0.0	7.6
	Pentacosene	2.3	2.4	2.5	2.8	1.5	2.7	3.0	2.5	1.9	2.9	3.5	2.6	1.7	2.5
	Pentacosane	5.6	9.0	13.1	18.7	0.0	9.4	9.0	0.0	0.0	9.1	0.0	9.4	0.0	7.7
	3 Me C25	0.0	5.7	6.9	6.3	6.0	5.5	5.4	8.1	7.9	4.8	7.1	5.1	8.1	6.4
	Heptacosene	4.2	4.6	4.6	5.6	3.6	4.5	5.8	3.4	3.2	5.7	5.4	4.9	3.2	4.0
	Heptacosane	0.0	10.1	0.0	28.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.5	0.0	0.0
	3 Me C27	5.3	7.8	8.2	12.1	9.7	11.1	7.6	0.0	9.9	9.7	0.0	6.5	11.5	11.9
	Nonacosene	0.0	12.7	0.0	12.4	0.0	0.0	13.7	0.0	0.0	0.0	0.0	15.5	0.0	12.0
Percentage of M+10	3 Me C23	0.0	0.0	0.0	4.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.0
	Pentacosene	1.6	1.6	1.9	2.0	1.1	1.7	2.2	2.0	1.5	2.1	2.2	2.1	1.4	1.7
	Pentacosane	0.0	4.7	9.4	14.3	0.0	0.0	5.6	0.0	0.0	6.7	0.0	6.3	0.0	5.1
	3 Me C25	0.0	3.2	4.7	4.4	4.6	4.6	4.6	0.0	5.3	3.3	0.0	3.7	5.7	5.2
	Heptacosene	3.2	3.3	3.3	4.0	2.5	3.1	4.2	3.1	2.4	4.0	3.5	3.7	2.3	3.2
	Heptacosane	0.0	0.0	0.0	21.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	3 Me C27	0.0	5.9	0.0	9.0	8.0	7.4	5.8	0.0	6.2	0.0	0.0	6.0	10.2	10.5
	Nonacosene	0.0	0.0	0.0	10.7	0.0	0.0	13.2	0.0	0.0	0.0	0.0	10.8	0.0	9.6

0
0-20
20-40
40-60
60-80
80-100
100+

Figure C.14: Data sheet showing page four of the raw substrate data for the L-leucine $^{13}C_6$,^{15}N experiment and *Formica lemani*. Please note that this data set extends across several pages.

L-Leucine (Negative Control)		*Myrmica sabuleti*														
		Con 1	Con 2	Con 3	Con 4	Con 5	Con 6	Con 7	Con 8	Con 9	Con 10	Con 11	Con 12	Con 13	Con 14	Con 15
Abundance of M	Tricosane	407	5483	4825	4135		4147	9297	4387	2056	5093	8072	2726	1395		5975
	5 Me C23	324	9138	6748	4353		3987	7955	2198	2209	3893	9934	2717	1085		6080
	Pentacosene	6967	36504	30056	25896		26624	65664	24880	19600	26424	68648	29888	16576		30936
	Pentacosane	777	2450	2310	2071		3404	5302	2830	1599	2467	4422	2328	1961		3039
	5 Me C25	6051	55432	43304	35472		38992	82552	27440	24040	35568	88816	36816	19448		44544
	Heptacosene	1144	11105	8252	7487		8087	17456	7017	4632	6927	17848	8981	3636		9151
	5 Me C27	988	13131	8559	7382		8182	15343	5331	4034	7105	17352	8193	3961		9761
Abundance of M+1	Tricosane		1478	1292	1031		1118	2555	1197	536	1342	1945	665	351		1540
	5 Me C23		1841	1343	889		793	1893	405	464	856	2092	522	281		1225
	Pentacosene	1979	10011	7908	6719		7051	17648	6835	5356	7322	17768	8060	4359		8413
	Pentacosane	208	662	609	553		872	1482	820	444	664	1201	638	600		834
	5 Me C25	1305	12031	9276	7971		8360	18456	5974	5407	7936	18944	7943	4183		9638
	Heptacosene	394	3193	2607	2114		2197	5017	2212	1338	2127	5379	2795	1133		2775
	5 Me C27	251	3283	2106	1696		2022	3589	1322	1004	1721	4451	2142	899		2382
Abundance of M+2	Tricosane		181	156				336			158	250				188
	5 Me C23		209					168				248				
	Pentacosene	240	1338	1058	888		962	2454	802	727	1000	2299	1123	581		1076
	Pentacosane							233								
	5 Me C25	166	1404	943	857		1013	2086	695	541	951	2091	948	478		1072
	Heptacosene		468	378	316		359	755	282	155	335	752	342	164		377
	5 Me C27		545	281	206		252	492	214		210	510	253			314
Abundance of M+3	Tricosane															
	5 Me C23															
	Pentacosene															
	Pentacosane															
	5 Me C25															
	Heptacosene															
	5 Me C27															

Figure C.15: Data sheet showing page one of the raw control data for the L-leucine $^{13}C_6$,^{15}N experiment and *Myrmica sabuleti*.

L-Leucine (Negative Control)		*Myrmica sabuleti*														
		Con 1	Con 2	Con 3	Con 4	Con 5	Con 6	Con 7	Con 8	Con 9	Con 10	Con 11	Con 12	Con 13	Con 14	Con 15
Percentage of M+1	Tricosane	0.0	27.0	26.8	24.9		27.0	27.5	27.3	26.1	26.3	24.1	24.4	25.2		25.8
	5 Me C23	0.0	20.1	19.9	20.4		19.9	23.8	18.4	21.0	22.0	21.1	19.2	25.9		20.1
	Pentacosene	28.4	27.4	26.3	25.9		26.5	26.9	27.5	27.3	27.7	25.9	27.0	26.3		27.2
	Pentacosane	26.8	27.0	26.4	26.7		25.6	28.0	29.0	27.8	26.9	27.2	27.4	30.6		27.4
	5 Me C25	21.6	21.7	21.4	22.5		21.4	22.4	21.8	22.5	22.3	21.3	21.6	21.5		21.6
	Heptacosene	34.4	28.8	31.6	28.2		27.2	28.7	31.5	28.9	30.7	30.1	31.1	31.2		30.3
	5 Me C27	25.4	25.0	24.6	23.0		24.7	23.4	24.8	24.9	24.2	25.7	26.1	22.7		24.4
Percentage of M+2	Tricosane	0.0	3.3	3.2	0.0		0.0	3.6	0.0	0.0	3.1	3.1	0.0	0.0		3.1
	5 Me C23	0.0	2.3	0.0	0.0		0.0	2.1	0.0	0.0	0.0	2.5	0.0	0.0		0.0
	Pentacosene	3.4	3.7	3.5	3.4		3.6	3.7	3.2	3.7	3.8	3.3	3.8	3.5		3.5
	Pentacosane	0.0	0.0	0.0	0.0		0.0	4.4	0.0	0.0	0.0	0.0	0.0	0.0		0.0
	5 Me C25	2.7	2.5	2.2	2.4		2.6	2.5	2.5	2.3	2.7	2.4	2.6	2.5		2.4
	Heptacosene	0.0	4.2	4.6	4.2		4.4	4.3	4.0	3.3	4.8	4.2	3.8	4.5		4.1
	5 Me C27	0.0	4.2	3.3	2.8		3.1	3.2	4.0	0.0	3.0	2.9	3.1	0.0		3.2
Percentage of M+3	Tricosane	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.0
	5 Me C23	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.0
	Pentacosene	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.0
	Pentacosane	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.0
	5 Me C25	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.0
	Heptacosene	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.0
	5 Me C27	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.0

Figure C.16: Data sheet showing page two of the raw control data for the L-leucine $^{13}C_6$,^{15}N experiment and *Myrmica sabuleti*.

L-Leucine $^{13}C_6$, ^{15}N		*Myrmica sabuleti*																
		Leu 1	Leu 2	Leu 3	Leu 4	Leu 5	Leu 6	Leu 7	Leu 8	Leu 9	Leu 10	Leu 11	Leu 12	Leu 13	Leu 14	Leu 15	Leu 16	Leu 17
Abundance of M	Tricosane	533	283	261	563	527	478	667		587		484	294		236	1401		293
	5 Me C23	866	375	467	3818	1908	924	2422		4558		486	997		726	7281		517
	Pentacosene	4141	2629	2435	5800	7785	6120	9203		6109		2525	3519		3326	19704		2874
	Pentacosane	393	320	256	376	568	509	545		510		461	245		200	913		195
	5 Me C23	8038	4641	5015	18376	18224	12045	17068		17896		6027	7605		6601	46696		5772
	Heptacosene	1109	553	599	2643	2391	1370	2540		3175		629	1016		730	7145		529
	5 Me C27	1138	566	558	2970	1773	1115	2397		3996		774	867		760	7244		584
Abundance of M+1	Tricosane	248			289	245	257	374		265		168	186		222	769		154
	5 Me C23	348		150	1311	757	421	1060		1708		177	428		346	2956		178
	Pentacosene	1637	846	905	1966	2644	2340	3818		2100		928	1334		1347	6857		994
	Pentacosane	184			185	190	267	332		207		157	151		152	423		198
	5 Me C23	3107	1512	2054	6283	7539	4872	7578		6752		2295	3279		3073	17464		2058
	Heptacosene	468	167	247	917	909	601	926		1130		240	326		267	2568		178
	5 Me C27	519	208	290	1163	889	732	1140		1836		388	434		319	3203		249
Abundance of M+2	Tricosane	237			357	374	368	585		360		243	228		321	935		236
	5 Me C23	319		165	979	730	372	992		1479		171	377		336	2282		157
	Pentacosene	1014	424	616	1012	1753	1652	2355		1199		601	931		976	4124		520
	Pentacosane	209			155	249	292	299				165	249		260	403		229
	5 Me C23	2772	1282	1866	4568	7006	4750	6989		5809		1983	2888		2993	14227		1389
	Heptacosene	212			396	409	435	556		613		178	180		188	1243		
	5 Me C27	408	170	246	852	946	603	1014		1688		307	367		381	2805		162
Abundance of M+3	Tricosane	314		162	514	528	613	820		471		375	373		457	1171		319
	5 Me C23	360		229	1114	796	442	1168		1886		240	436		478	2384		176
	Pentacosene	1026	436	772	1137	1800	2123	2907		1340		728	1062		1261	3938		631
	Pentacosane	288			221	419	403	395		219		354	345		284	466		221
	5 Me C23	3511	1441	2404	5459	9130	6218	9241		7613		2512	3541		3741	14774		1896
	Heptacosene	266			451	503	585	667		585		228	278		247	1185		
	5 Me C27	644	241	350	1144	1474	1102	1492		2200		396	568		468	3343		221
Abundance of M+4	Tricosane	515	160	225	761	829	895	1089		782		658	532		499	2033		374
	5 Me C23	419		260	1377	1195	481	1530		2357		301	481		488	2457		180
	Pentacosene	1532	621	930	1583	3038	3029	3931		1789		1028	1445		1702	5215		965
	Pentacosane	383			425	729	586	578		340		533	394		340	636		305
	5 Me C23	4421	2028	3089	6744	12147	7706	10618		9573		3238	4584		4335	16464		2160
	Heptacosene	354		196	614	835	819	1025		745		352	356		286	1569		161
	5 Me C27	889	281	456	1593	2039	1311	2211		3012		658	750		626	3761		292
Abundance of M+5	Tricosane	692	189	315	1036	1270	1305	1488		1128		920	655		652	2093		431
	5 Me C23	522	172	273	1453	1372	551	1524		2489		381	567		538	2426		221
	Pentacosene	2009	875	1350	2062	4203	3819	4817		2328		1537	1967		2017	5824		1318
	Pentacosane	499		189	497	934	858	841		530		689	490		347	768		338
	5 Me C23	5009	2230	3636	7575	14536	8716	12797		11369		4101	5042		4877	16840		2468
	Heptacosene	526	163	331	759	1144	1159	1332		1109		461	459		455	1705		211
	5 Me C27	987	376	583	1839	2606	1554	2283		3830		755	856		680	4212		415

Figure C.17: Data sheet showing page one of the raw substrate data for the L-leucine $^{13}C_6$,^{15}N experiment and *Myrmica sabuleti*.

L-Leucine $^{13}C_6$, ^{15}N		*Myrmica sabuleti*																
		Leu 1	Leu 2	Leu 3	Leu 4	Leu 5	Leu 6	Leu 7	Leu 8	Leu 9	Leu 10	Leu 11	Leu 12	Leu 13	Leu 14	Leu 15	Leu 16	Leu 17
Abundance of M+6	Tricosane	846	218	501	1260	1723	1442	1902		1455		1245	766		691	2291		536
	5 Me C23	471	164	304	1465	1517	620	1515		2736		377	651		492	2238		200
	Pentacosene	2480	1202	1733	2407	5547	4918	5891		3004		1917	2293		2322	6400		1615
	Pentacosane	603		262	655	1117	978	1110		751		950	566		375	1039		346
	5 Me C23	5270	2522	3815	7769	16150	9350	13600		12067		4491	5346		4789	16137		2860
	Heptacosene	635	258	395	1082	1590	1617	1666		1355		630	571		489	2137		266
	5 Me C27	1196	451	717	2057	3032	2123	2622		4329		998	946		731	4486		387
Abundance of M+7	Tricosane	1023	296	442	1400	2159	1746	1903		1777		1524	883		666	2287		506
	5 Me C23	476	177	277	1356	1287	632	1481		2563		352	497		360	1894		208
	Pentacosene	2898	1416	2049	2929	6742	5745	6971		3741		2669	2604		2475	7390		1878
	Pentacosane	620	183	325	724	1362	1208	1134		908		1067	607		324	1023		258
	5 Me C23	5265	2675	3909	7405	16448	9003	13052		12575		4485	5190		4366	14532		2759
	Heptacosene	862	351	515	1196	2161	1854	1830		1754		804	695		601	2400		380
	5 Me C27	1222	496	597	2216	3577	2107	2735		4803		1091	910		711	4229		461
Abundance of M+8	Tricosane	1078	329	613	1430	2629	1976	2095		1797		1885	978		678	2032		586
	5 Me C23	350	161	254	1134	1300	484	1136		2306		324	486		318	1593		
	Pentacosene	3230	1678	2418	3213	7876	6534	7260		4110		2976	2955		2718	7843		2251
	Pentacosane	683	310	387	817	1452	1286	1088		1115		1157	495		372	1091		241
	5 Me C23	4594	1856	3457	6552	15087	8777	11528		11545		4509	4652		3953	12718		2506
	Heptacosene	1010	443	595	1378	2589	2260	2150		1973		931	843		620	2463		453
	5 Me C27	1147	575	716	2238	3389	2247	2820		5089		997	891		747	3744		426
Abundance of M+9	Tricosane	1093	344	631	1427	2530	1870	2115		1951		1889	1060		594	1737		421
	5 Me C23	285		208	907	1034	395	1019		1871		298	338		245	1161		
	Pentacosene	3198	1885	2450	3211	8429	6392	7238		4410		3274	3077		2635	7716		2231
	Pentacosane	612	302	369	860	1615	1393	1074		1209		1220	550		344	1120		185
	5 Me C23	3589	1404	3045	5358	12909	7475	10464		9985		4033	3853		3055	10302		2236
	Heptacosene	118	472	718	1649	3013	2464	2317		2253		989	933		745	2644		502
	5 Me C27	1075	625	571	1969	3456	2178	2379		4602		1113	850		605	3494		423
Abundance of M+10	Tricosane	874	360	537	1264	2610	1827	2013		1866		1804	940		488	1508		387
	5 Me C23	179		166	546	726	274	677		1331		240	263		196	758		
	Pentacosene	3036	2001	2474	3331	8976	6844	7505		4745		3450	2916		2296	7494		2220
	Pentacosane	580	388	382	770	1387	1366	1274		1121		1165	434		229	1130		163
	5 Me C23	2955	950	2426	4032	10230	5734	7920		8249		2999	3003		2265	7321		1783
	Heptacosene	1101	513	712	1593	3194	2294	2263		2501		1104	904		722	2794		509
	5 Me C27	923	540	547	1608	2965	1777	2139		3986		957	700		503	2836		325
Abundance of M+11	Tricosane	772	296	465	1070	2141	1669	1547		1757		1707	773		434	1123		264
	5 Me C23				400	367	171	455		941			200			501		
	Pentacosene	2892	1945	2401	315	8599	5822	6509		4098		3213	1809		2053	6459		1926
	Pentacosane	452	346	292	656	1315	1139	1007		1131		925	407		202	949		166
	5 Me C23	1892	745	1763	2852	7205	4076	5484		5780		2237	2162		1583	5203		1278
	Heptacosene	953	571	702	1550	3309	2262	1852		2466		1045	895		666	2428		419
	5 Me C27	683	460	426	1239	2436	1500	1535		3350		800	660		418	2257		279
Abundance of M+12	Tricosane	610	284	420	902	1735	1222	1314		1379		1453	698		318	720		189
	5 Me C23				233	260		290		508						349		
	Pentacosene	2449	1751	2074	2431	7389	5242	5599		3874		2790	2312		1867	5862		1602
	Pentacosane	344	386	242	594	982	1201	888		926		715	291			711		
	5 Me C23	1314	578	1082	1775	5094	2802	3736		4158		1559	1329		980	3451		841
	Heptacosene	956	569	652	1334	3136	2158	1933		2085		935	845		592	2229		305
	5 Me C27	483	276	345	887	1779	1082	1300		2717		569	395		312	1597		255

Figure C.18: Data sheet showing page two of the raw substrate data for the L-leucine $^{13}C_6$, ^{15}N experiment and *Myrmica sabuleti*.

L-Leucine $^{13}C_6$, ^{15}N		*Myrmica sabuleti*																
		Leu 1	Leu 2	Leu 3	Leu 4	Leu 5	Leu 6	Leu 7	Leu 8	Leu 9	Leu 10	Leu 11	Leu 12	Leu 13	Leu 14	Leu 15	Leu 16	Leu 17
Abundance of M+13	Tricosane	452	195	358	590	1380	1052	1046		1112		1088	525		235	554		
	5 Me C23				246	165		204		436						481		
	Pentacosene	1808	1463	1714	1924	6207	4095	4563		3257		2433	1936		1448	4513		1300
	Pentacosane	235	239	182	448	851	958	756		881		483	250			635		
	5 Me C23	1062	280	820	1696	3386	2018	2960		3135		1175	1086		879	3630		746
	Heptacosene	811	470	654	1235	2761	1905	1525		2012		739	735		540	1791		294
	5 Me C27	352	268	242	803	1422	769	928		1946		439	338		198	1416		203
Abundance of M+14	Tricosane	305	178	223	404	1000	722	707		898		800	404			288		
	5 Me C23				250	159		214		440						491		
	Pentacosene	1388	1154	1357	1480	4755	3259	3359		2403		1897	1523		1114	3546		882
	Pentacosane	190	219		303	562	958	521		589		332				403		
	5 Me C23	788	168	726	1533	2562	1558	2378		2479		917	945		711	3881		621
	Heptacosene	671	404	475	1014	2457	1720	1124		1738		647	586		487	1614		199
	5 Me C27	248	177	169	572	899	583	671		1322		296	244		186	1080		
Abundance of M+15	Tricosane	185			247	667	486	487		555		528	240			190		
	5 Me C23					256										205		
	Pentacosene	973	824	905	1070	3504	2321	2334		1967		1444	946		759	2645		649
	Pentacosane		181		251	425	604	408		427		203				376		
	5 Me C23	374	327	279	604	1116	658	983		1106		416	407		322	1704		262
	Heptacosene	472	346	379	778	1890	1138	887		1335		447	458		285	1160		155
	5 Me C27				305	446	367	344		837		166				500		
Percentage of M+1	Tricosane	46.5	0.0	0.0	51.3	46.5	53.8	56.1		45.1		34.7	63.3		94.1	54.9		52.6
	5 Me C23	40.2	0.0	32.1	34.3	39.7	45.6	43.8		37.5		36.4	42.9		47.7	40.6		34.4
	Pentacosene	39.5	32.2	37.2	33.9	34.0	38.2	41.5		34.4		36.8	37.9		40.5	34.8		34.6
	Pentacosane	46.8	0.0	0.0	49.2	33.5	52.5	60.9		40.6		34.1	61.6		76.0	46.3		101.5
	5 Me C23	38.7	32.6	41.0	34.2	41.4	40.4	44.4		37.7		38.1	43.1		46.6	37.4		35.7
	Heptacosene	42.2	30.2	41.2	34.7	38.0	43.9	36.5		35.6		38.2	32.1		36.6	35.9		33.6
	5 Me C27	45.6	36.7	52.0	39.2	50.1	65.7	47.6		45.9		50.1	50.1		42.0	44.2		42.6
Percentage of M+2	Tricosane	44.5	0.0	0.0	63.4	71.0	77.0	87.7		61.3		50.2	77.6		136.0	66.7		80.5
	5 Me C23	36.8	0.0	35.3	25.6	38.3	40.3	41.0		32.4		35.2	37.8		46.3	31.3		30.4
	Pentacosene	24.5	16.1	25.3	17.4	22.5	27.0	25.6		19.6		23.8	26.5		29.3	20.9		18.1
	Pentacosane	53.2	0.0	0.0	41.2	43.8	57.4	54.9		0.0		35.8	101.6		130.0	44.1		117.4
	5 Me C23	34.5	27.6	37.2	24.9	38.4	39.4	40.9		32.5		32.9	38.0		45.3	30.5		24.1
	Heptacosene	19.1	0.0	0.0	15.0	17.1	31.8	21.9		19.3		28.3	17.7		25.8	17.4		0.0
	5 Me C27	35.9	30.0	44.1	28.7	53.4	54.1	42.3		42.2		39.7	42.3		50.1	38.7		27.7
Percentage of M+3	Tricosane	58.9	0.0	62.1	91.3	100.2	128.2	122.9		80.2		77.5	126.9		193.6	83.6		108.9
	5 Me C23	41.6	0.0	49.0	29.2	41.7	47.8	48.2		41.4		49.4	43.7		65.8	32.7		34.0
	Pentacosene	24.8	16.6	31.7	19.6	23.1	34.7	31.6		21.9		28.8	30.2		37.9	20.0		22.0
	Pentacosane	73.3	0.0	0.0	58.8	73.8	79.2	72.5		42.9		76.8	140.8		142.0	51.0		113.3
	5 Me C23	43.7	31.0	47.9	29.7	50.1	51.6	54.1		42.5		41.7	46.6		56.7	31.6		32.8
	Heptacosene	24.0	0.0	0.0	17.1	21.0	42.7	26.3		18.4		36.2	27.4		33.8	16.6		0.0
	5 Me C27	56.6	42.6	62.7	38.5	83.1	98.8	62.2		55.1		51.2	65.5		61.6	46.1		37.8
Percentage of M+4	Tricosane	96.6	56.5	86.2	135.2	157.3	187.2	163.3		133.2		136.0	181.0		211.4	145.1		127.6
	5 Me C23	48.4	0.0	55.7	36.1	62.6	52.1	63.2		51.7		61.9	48.2		67.2	33.7		34.8
	Pentacosene	37.0	23.6	38.2	27.3	39.0	49.5	42.7		29.3		40.7	41.1		51.2	26.5		33.6
	Pentacosane	97.5	0.0	0.0	113.0	128.3	115.1	106.1		66.7		115.6	160.8		170.0	69.7		156.4
	5 Me C23	55.0	43.7	61.6	36.7	66.7	64.0	62.2		53.5		53.7	60.3		65.7	35.3		37.4
	Heptacosene	31.9	0.0	32.7	23.2	34.9	59.8	40.4		23.5		56.0	35.0		39.2	22.0		30.4
	5 Me C27	78.1	49.6	81.7	53.6	115.0	117.6	92.2		75.4		85.0	86.5		82.4	51.9		50.0

0
0-20
20-40
40-60
80-100
100+

Figure C.19: Data sheet showing page three of the raw substrate data for the L-leucine $^{13}C_6$,^{15}N experiment and *Myrmica sabuleti*.

L-Leucine $^{13}C_6$, ^{15}N		*Myrmica sabuleti*																
		Leu 1	Leu 2	Leu 3	Leu 4	Leu 5	Leu 6	Leu 7	Leu 8	Leu 9	Leu 10	Leu 11	Leu 12	Leu 13	Leu 14	Leu 15	Leu 16	Leu 17
Percentage of M+5	Tricosane	129.8	66.8	120.7	184.0	241.0	273.0	223.1		192.2		190.1	222.8		276.3	149.4		147.1
	5 Me C23	60.3	45.9	58.5	38.1	71.9	59.6	62.9		54.6		78.4	56.9		74.1	33.3		42.7
	Pentacosene	48.5	33.3	55.4	35.6	54.0	62.4	52.3		38.1		60.9	55.9		60.6	29.6		45.9
	Pentacosane	127.0	0.0	73.8	132.2	164.4	168.6	154.3		103.9		149.5	200.0		173.5	84.1		173.3
	5 Me C23	62.3	48.0	72.5	41.2	79.8	72.4	75.0		63.5		68.0	66.3		73.9	36.1		42.8
	Heptacosene	47.4	29.5	55.3	28.7	47.8	84.6	52.4		34.9		73.3	45.2		62.3	23.9		39.9
	5 Me C27	86.7	66.4	104.5	61.9	147.0	139.4	95.2		95.8		97.5	98.7		89.5	58.1		71.1
Percentage of M+6	Tricosane	158.7	77.0	192.0	223.8	326.9	301.7	285.2		247.9		257.2	260.5		292.8	163.5		182.9
	5 Me C23	54.4	43.7	65.1	38.4	79.5	67.1	62.6		60.0		77.6	65.3		67.8	30.7		38.7
	Pentacosene	59.9	45.7	71.2	41.5	71.3	80.4	64.0		49.2		75.9	65.2		69.8	32.5		56.2
	Pentacosane	153.4	0.0	102.3	174.2	196.7	192.1	203.7		147.3		206.1	231.0		187.5	113.8		177.4
	5 Me C23	65.6	54.3	76.1	42.3	88.6	77.6	79.7		67.4		74.5	70.3		72.5	34.6		49.5
	Heptacosene	57.3	46.7	65.9	40.9	66.5	118.0	65.6		42.7		100.2	56.2		67.0	29.9		50.3
	5 Me C27	105.1	79.7	128.5	69.3	171.0	190.4	109.4		108.3		128.9	109.1		96.2	61.9		66.3
Percentage of M+7	Tricosane	191.9	104.6	169.3	248.7	409.7	365.3	285.3		302.7		314.9	300.3		282.2	163.2		172.7
	5 Me C23	55.0	47.2	59.3	35.5	67.5	68.4	61.1		56.2		72.4	49.8		49.6	26.0		40.2
	Pentacosene	70.0	53.9	84.1	50.5	86.6	93.9	75.7		61.2		105.7	74.0		74.4	37.5		65.3
	Pentacosane	157.8	57.2	127.0	192.6	239.8	237.3	208.1		178.0		231.5	247.8		162.0	112.0		132.3
	5 Me C23	65.5	57.6	77.9	40.3	90.3	74.7	76.5		70.3		74.4	68.2		66.1	31.1		47.8
	Heptacosene	77.7	63.5	86.0	45.3	90.4	135.3	72.0		55.2		127.8	68.4		82.3	33.6		71.8
	5 Me C27	107.4	87.6	107.0	74.6	201.7	189.0	114.1		120.2		141.0	105.0		93.6	58.4		78.9
Percentage of M+8	Tricosane	202.3	116.3	234.9	254.0	498.9	413.4	314.1		306.1		389.5	332.7		287.3	145.0		200.0
	5 Me C23	40.4	42.9	54.4	29.7	68.1	52.4	46.9		50.6		66.7	48.7		43.8	21.9		0.0
	Pentacosene	78.0	63.8	99.3	55.4	101.2	106.8	78.9		67.3		117.9	84.0		81.7	39.8		78.3
	Pentacosane	173.8	96.9	151.2	217.3	255.6	252.7	199.6		218.6		251.0	202.0		186.0	119.5		123.6
	5 Me C23	57.2	40.0	68.9	35.7	82.8	72.9	67.5		64.5		74.8	61.2		59.9	27.2		43.4
	Heptacosene	91.1	80.1	99.3	52.1	108.3	165.0	84.6		62.1		148.0	83.0		84.9	34.5		85.6
	5 Me C27	100.8	101.6	128.3	75.4	191.1	201.5	117.6		127.4		128.8	102.8		98.3	51.7		72.9
Percentage of M+9	Tricosane	205.1	121.6	241.8	253.5	480.1	391.2	317.1		332.4		390.3	360.5		251.7	124.0		143.7
	5 Me C23	32.9	0.0	44.5	23.8	54.2	42.7	42.1		41.0		61.3	33.9		33.7	15.9		0.0
	Pentacosene	77.2	71.7	100.6	55.4	108.3	104.4	78.6		72.2		129.7	87.4		79.2	39.2		77.6
	Pentacosane	155.7	94.4	144.1	228.7	284.3	273.7	197.1		237.1		264.6	224.5		172.0	122.7		94.9
	5 Me C23	44.7	30.3	60.7	29.2	70.8	62.1	61.3		55.8		66.9	50.7		46.3	22.1		38.7
	Heptacosene	10.6	85.4	119.9	62.4	126.0	179.9	91.2		71.0		157.2	91.8		102.1	37.0		94.9
	5 Me C27	94.5	110.4	102.3	66.3	194.9	195.3	99.2		115.2		143.8	98.0		79.6	48.2		72.4
Percentage of M+10	Tricosane	164.0	127.2	205.7	224.5	495.3	382.2	301.8		317.9		372.7	319.7		206.8	107.6		132.1
	5 Me C23	20.7	0.0	35.5	14.3	38.1	29.7	28.0		29.2		49.4	26.4		27.0	10.4		0.0
	Pentacosene	73.3	76.1	101.6	57.4	115.3	111.8	81.5		77.7		136.6	82.9		69.0	38.0		77.2
	Pentacosane	147.6	121.3	149.2	204.8	244.2	268.4	233.8		219.8		252.7	177.1		114.5	123.8		83.6
	5 Me C23	36.8	20.5	48.4	21.9	56.1	47.6	46.4		46.1		49.8	39.5		34.3	15.7		30.9
	Heptacosene	99.3	92.8	118.9	60.3	133.6	167.4	89.1		78.8		175.5	89.0		98.9	39.1		96.2
	5 Me C27	81.1	95.4	98.0	54.1	167.2	159.4	89.2		99.7		123.6	80.7		66.2	39.1		55.7
Percentage of M+11	Tricosane	144.8	104.6	178.2	190.1	406.3	349.2	231.9		299.3		352.7	262.9		183.9	80.2		90.1
	5 Me C23	0.0	0.0	0.0	10.5	19.2	18.5	18.8		20.6		0.0	20.1		0.0	6.9		0.0
	Pentacosene	69.8	74.0	98.6	5.4	110.5	95.1	70.7		67.1		127.2	51.4		61.7	32.8		67.0
	Pentacosane	115.0	108.1	114.1	174.5	231.5	223.8	184.8		221.8		200.7	166.1		101.0	103.9		85.1
	5 Me C23	23.5	16.1	35.2	15.5	39.5	33.8	32.1		32.3		37.1	28.4		24.0	11.1		22.1
	Heptacosene	85.9	103.3	117.2	58.6	138.4	165.1	72.9		77.7		166.1	88.1		91.2	34.0		79.2
	5 Me C27	60.0	81.3	76.3	41.7	137.4	134.5	64.0		83.8		103.4	76.1		55.0	31.2		47.8

0
0-20
20-40
40-60
80-100
100+

Figure C.20: Data sheet showing page four of the raw substrate data for the L-leucine $^{13}C_6$,^{15}N experiment and *Myrmica sabuleti*.

L-Leucine $^{13}C_6$, ^{15}N		*Myrmica sabuleti*																
		Leu 1	Leu 2	Leu 3	Leu 4	Leu 5	Leu 6	Leu 7	Leu 8	Leu 9	Leu 10	Leu 11	Leu 12	Leu 13	Leu 14	Leu 15	Leu 16	Leu 17
Percentage of M+12	Tricosane	114.4	100.4	160.9	160.2	329.2	255.6	197.0		234.9		300.2	237.4		134.7	51.4		64.5
	5 Me C23	0.0	0.0	0.0	6.1	13.6	0.0	12.0		11.1		0.0	0.0		0.0	4.8		0.0
	Pentacosene	59.1	66.6	85.2	41.9	94.9	85.7	60.8		63.4		110.5	65.7		56.1	29.8		55.7
	Pentacosane	87.5	120.6	94.5	158.0	172.9	236.0	162.9		181.6		155.1	118.8		0.0	77.9		0.0
	5 Me C23	16.3	12.5	21.6	9.7	28.0	23.3	21.9		23.2		25.9	17.5		14.8	7.4		14.6
	Heptacosene	86.2	102.9	108.8	50.5	131.2	157.5	76.1		65.7		148.6	83.2		81.1	31.2		57.7
	5 Me C27	42.4	48.8	61.8	29.9	100.3	97.0	54.2		68.0		73.5	45.6		41.1	22.0		43.7
Percentage of M+13	Tricosane	84.8	68.9	137.2	104.8	261.9	220.1	156.8		189.4		224.8	178.6		99.6	39.5		0.0
	5 Me C23	0.0	0.0	0.0	6.4	8.6	0.0	8.4		9.6		0.0	0.0		0.0	6.6		0.0
	Pentacosene	43.7	55.6	70.4	33.2	79.7	66.9	49.6		53.3		96.4	55.0		43.5	22.9		45.2
	Pentacosane	59.8	74.7	71.1	119.1	149.8	188.2	138.7		172.7		104.8	102.0		0.0	69.6		0.0
	5 Me C23	13.2	6.0	16.4	9.2	18.6	16.8	17.3		17.5		19.5	14.3		13.3	7.8		12.9
	Heptacosene	73.1	85.0	109.2	46.7	115.5	139.1	60.0		63.4		117.5	72.3		74.0	25.1		55.6
	5 Me C27	30.9	47.3	43.4	27.0	80.2	69.0	38.7		48.7		56.7	39.0		26.1	19.5		34.8
Percentage of M+14	Tricosane	57.2	62.9	85.4	71.8	189.8	151.0	106.0		153.0		165.3	137.4		0.0	20.6		0.0
	5 Me C23	0.0	0.0	0.0	6.5	8.3	0.0	8.8		9.7		0.0	0.0		0.0	6.7		0.0
	Pentacosene	33.5	43.9	55.7	25.5	61.1	53.3	36.5		39.3		75.1	43.3		33.5	18.0		30.7
	Pentacosane	48.3	68.4	0.0	80.6	98.9	188.2	95.6		115.5		72.0	0.0		0.0	44.1		0.0
	5 Me C23	9.8	3.6	14.5	8.3	14.1	12.9	13.9		13.9		15.2	12.4		10.8	8.3		10.8
	Heptacosene	60.5	73.1	79.3	38.4	102.8	125.5	44.3		54.7		102.9	57.7		66.7	22.6		37.6
	5 Me C27	21.8	31.3	30.3	19.3	50.7	52.3	28.0		33.1		38.2	28.1		24.5	14.9		0.0
Percentage of M+15	Tricosane	34.7	0.0	0.0	43.9	126.6	101.7	73.0		94.5		109.1	81.6		0.0	13.6		0.0
	5 Me C23	0.0	0.0	0.0	0.0	13.4	0.0	0.0		0.0		0.0	0.0		0.0	2.8		0.0
	Pentacosene	23.5	31.3	37.2	18.4	45.0	37.9	25.4		32.2		57.2	26.9		22.8	13.4		22.6
	Pentacosane	0.0	56.6	0.0	66.8	74.8	118.7	74.9		83.7		44.0	0.0		0.0	41.2		0.0
	5 Me C23	4.7	7.0	5.6	3.3	6.1	5.5	5.8		6.2		6.9	5.4		4.9	3.6		4.5
	Heptacosene	42.6	62.6	63.3	29.4	79.0	83.1	34.9		42.0		71.1	45.1		39.0	16.2		29.3
	5 Me C27	0.0	0.0	0.0	10.3	25.2	32.9	14.4		20.9		21.4	0.0		0.0	6.9		0.0

0
0-20
20-40
40-60
80-100
100+

Figure C.21: Data sheet showing page five of the raw substrate data for the L-leucine $^{13}C_6$, ^{15}N experiment and *Myrmica sabuleti*.

DL-Valine (Negative Control)		*Formica lemani*													
		Con 1	Con 2	Con 3	Con 4	Con 5	Con 6	Con 7	Con 8	Con 9	Con 10	Con 11	Con 12	Con 13	Con 14
Abundance of M	Tricosene	5049	6413	4377	7559	5106	5243	9874	13978	13635	7189	4460	6422	4029	7990
	Tricosane	1549	1982	2954	3211	2491	2272	3754	2366	2148	1371	2421	1874	2465	1702
	3 Me C23	7927	8867	5067	7357	9382	6736	10459	14725	16992	10426	7065	10229	3372	9989
	Pentacosene	109432	137472	116792	139776	179712	155584	178432	236288	248128	172480	101896	163264	80528	137152
	Pentacosane	2850	2052	5149	7119	4487	7220	8256	8619	4299	2629	5124	4427	2297	4162
	3 Me C25	30464	35800	24592	29328	39664	38448	28152	57656	57544	44400	28008	41880	21000	31504
	Heptacosene	36416	43560	33680	51168	55688	49240	49352	66696	67832	47792	35064	48448	31048	40456
	Heptacosane		1676	1201	2301	1097	1065	996	1931	551	345	921	1029	583	837
Abundance of M+1	Tricosene	1351	2400	949	1538	1033	1039	1902	4400	4201	4186	713	1820	1221	890
	Tricosane	159	1527		1146	1116		642	1629	1653	507	616	351	247	
	3 Me C23	1168	1107	1585	2825	1517	4649	2778	2964	2854	2082	1887	2489	1225	560
	Pentacosene	21920	42048	32512	37152	44040	34992	46080	66984	68832	45928	29112	41256	25216	41776
	Pentacosane	1341	1576	2523	2692	795	1879	2702	1538	2324	621	548	2423	339	1854
	3 Me C25	6421	12306	5607	6713	9810	10028	10762	13203	9433	7704	6178	11625	4258	5624
	Heptacosene	9673	10066	10888	11203	15488	17968	10251	16648	18192	13520	10314	16568	7955	16480
	Heptacosane			702	1161	238	908	430	930	187					
Abundance of M+2	Tricosene				506	181		350		557					411
	Tricosane	443	384			369				565			182		
	3 Me C23		230	289		698		262	1004	933	433		209		
	Pentacosene	3554	6190	3142	4068	7700	5201	5102	7478	9305	5206	3060	5239	3811	3651
	Pentacosane			265	163	636	344	363	704				371		908
	3 Me C25		620	1223	1154	2320	733	1751	2961	2228	743	605	1380	991	788
	Heptacosene	757	1694	2425	834	1818	533	3151	2656	3829	2983		2423	1059	2257
	Heptacosane						221	239							
Abundance of M+3	Tricosene				382			165		181					
	Tricosane		211												
	3 Me C23								184						
	Pentacosene	246	542				1445	1500	1201	1699	379		677		
	Pentacosane			668			1023	334					315		
	3 Me C25	667							430				669		
	Heptacosene		417	162			241	455		220					
	Heptacosane														

Figure C.22: Data sheet showing page one of the raw control data for the DL-valine d_8 experiment and *Formica lemani*.

DL-Valine (Negative Control)		*Formica lemani*													
		Con 15	Con 16	Con 17	Con 18	Con 19	Con 20	Con 21	Con 22	Con 23	Con 24	Con 25	Con 26	Con 27	Con 28
Abundance of M	Tricosene	4911	10050	2605	6159	7801	5626	5573	4759	3683	7182	5678	9703	6450	6158
	Tricosane	2594	3195	2100	662	1791	1173	2035	1105	645	1553	1595	1677	4259	426
	3 Me C23	8895	9169	8589	6877	7549	6250	5254	7873	6568	6884	5602	9642	7958	7480
	Pentacosene	125552	173056	106184	96104	104776	123648	97256	97520	80952	148608	129536	259200	166976	116680
	Pentacosane	1070	6323	4496	3360	5023	4690	2762	2615	1822	4298	2889	3080	3680	5282
	3 Me C25	28328	41624	29544	19456	29072	28896	21760	24024	21728	34248	36720	55544	40192	32968
	Heptacosene	33888	48488	36760	30424	35496	32000	30352	30944	26720	44792	46672	83096	51232	30312
	Heptacosane	594	1877	924	447	625	1107	2413	346	278	2336	1124	1096	307	519
Abundance of M+1	Tricosene	2395	1629	817	183	1233	1465	1719	1920	700	2196	1053	3158	2692	1373
	Tricosane	717	1627	261	239			358		225	201	802	167	823	
	3 Me C23	2034	1453	1740	809	1894	763	1355	2429	1354	2263	2723	3105	2915	1304
	Pentacosene	31672	39008	32784	26616	35056	28536	20512	31120	18744	37424	35896	76720	43992	28792
	Pentacosane	382	2153	1570	1147	1276	1729	1032		926	1665	1400	236	1702	521
	3 Me C25	6366	8778	7366	5262	5923	7350	8139	10352	5974	8042	7495	13838	6761	6994
	Heptacosene	11960	12716	9007	6668	8301	9938	5795	6539	7430	13216	9256	15825	15236	9928
	Heptacosane		613	458	267						256				
Abundance of M+2	Tricosene					664	495				864		944		
	Tricosane														
	3 Me C23			245			341						807		458
	Pentacosene	17498	7144	3278	2635	4331	3917	4140	3328	2544	4092	5613	9868	6592	2007
	Pentacosane							361							
	3 Me C25	1612	1290	518	564	482	1286	455	2147	1255	1836	228	2413	1964	1607
	Heptacosene	2297	2679	2512	1098	1640	357	1389	1429	1211	3756	3406	2423	559	190
	Heptacosane														
Abundance of M+3	Tricosene														
	Tricosane														
	3 Me C23														
	Pentacosene		451		155			571			3658	304	557	1249	609
	Pentacosane														
	3 Me C25	315	676												
	Heptacosene									327					
	Heptacosane														

Figure C.23: Data sheet showing page two of the raw control data for the DL-valine d_8 experiment and *Formica lemani*.

DL-Valine (Negative Control)		*Formica lemani*													
		Con 1	Con 2	Con 3	Con 4	Con 5	Con 6	Con 7	Con 8	Con 9	Con 10	Con 11	Con 12	Con 13	Con 14
Percentage of M+1	Tricosene	26.8	37.4	21.7	20.3	20.2	19.8	19.3	31.5	30.8	58.2	16.0	28.3	30.3	11.1
	Tricosane	10.3	77.0	0.0	35.7	44.8	0.0	17.1	68.9	77.0	37.0	25.4	18.7	10.0	0.0
	3 Me C23	14.7	12.5	31.3	38.4	16.2	69.0	26.6	20.1	16.8	20.0	26.7	24.3	36.3	5.6
	Pentacosene	20.0	30.6	27.8	26.6	24.5	22.5	25.8	28.3	27.7	26.6	28.6	25.3	31.3	30.5
	Pentacosane	47.1	76.8	49.0	37.8	17.7	26.0	32.7	17.8	54.1	23.6	10.7	54.7	14.8	44.5
	3 Me C25	21.1	34.4	22.8	22.9	24.7	26.1	38.2	22.9	16.4	17.4	22.1	27.8	20.3	17.9
	Heptacosene	26.6	23.1	32.3	21.9	27.8	36.5	20.8	25.0	26.8	28.3	29.4	34.2	25.6	40.7
	Heptacosane		0.0	58.5	50.5	21.7	85.3	43.2	48.2	33.9	0.0	0.0	0.0	0.0	0.0
Percentage of M+2	Tricosene	0.0	0.0	0.0	6.7	3.5	0.0	3.5	0.0	4.1	0.0	0.0	0.0	0.0	5.1
	Tricosane	28.6	19.4	0.0	0.0	14.8	0.0	0.0	0.0	26.3	0.0	0.0	9.7	0.0	0.0
	3 Me C23	0.0	2.6	5.7	0.0	7.4	0.0	2.5	6.8	5.5	4.2	0.0	2.0	0.0	0.0
	Pentacosene	3.2	4.5	2.7	2.9	4.3	3.3	2.9	3.2	3.8	3.0	3.0	3.2	4.7	2.7
	Pentacosane	0.0	0.0	5.1	2.3	14.2	4.8	4.4	8.2	0.0	0.0	0.0	8.4	0.0	21.8
	3 Me C25	0.0	1.7	5.0	3.9	5.8	1.9	6.2	5.1	3.9	1.7	2.2	3.3	4.7	2.5
	Heptacosene	2.1	3.9	7.2	1.6	3.3	1.1	6.4	4.0	5.6	6.2	0.0	5.0	3.4	5.6
	Heptacosane		0.0	0.0	0.0	0.0	20.8	24.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Percentage of M+3	Tricosene	0.0	0.0	0.0	5.1	0.0	0.0	1.7	0.0	1.3	0.0	0.0	0.0	0.0	0.0
	Tricosane	0.0	10.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	3 Me C23	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.2	0.0	0.0	0.0	0.0	0.0	0.0
	Pentacosene	0.2	0.4	0.0	0.0	0.0	0.9	0.8	0.5	0.7	0.2	0.0	0.4	0.0	0.0
	Pentacosane	0.0	0.0	13.0	0.0	0.0	14.2	4.0	0.0	0.0	0.0	0.0	7.1	0.0	0.0
	3 Me C25	2.2	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	1.6	0.0	0.0
	Heptacosene	0.0	1.0	0.5	0.0	0.0	0.5	0.9	0.0	0.3	0.0	0.0	0.0	0.0	0.0
	Heptacosane		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

0
0-20
20-40
40-60
60-80
80-100
100+

Figure C.24: Data sheet showing page three of the raw control data for the DL-valine d_8 experiment and *Formica lemani*.

DL-Valine (Negative Control)		*Formica lemani*													
		Con 15	Con 16	Con 17	Con 18	Con 19	Con 20	Con 21	Con 22	Con 23	Con 24	Con 25	Con 26	Con 27	Con 28
Percentage of M+1	Tricosene	48.8	16.2	31.4	3.0	15.8	26.0	30.8	40.3	19.0	30.6	18.5	32.5	41.7	22.3
	Tricosane	27.6	50.9	12.4	36.1	0.0	0.0	17.6	0.0	34.9	12.9	50.3	10.0	19.3	0.0
	3 Me C23	22.9	15.8	20.3	11.8	25.1	12.2	25.8	30.9	20.6	32.9	48.6	32.2	36.6	17.4
	Pentacosene	25.2	22.5	30.9	27.7	33.5	23.1	21.1	31.9	23.2	25.2	27.7	29.6	26.3	24.7
	Pentacosane	35.7	34.1	34.9	34.1	25.4	36.9	37.4	0.0	50.8	38.7	48.5	7.7	46.3	9.9
	3 Me C25	22.5	21.1	24.9	27.0	20.4	25.4	37.4	43.1	27.5	23.5	20.4	24.9	16.8	21.2
	Heptacosene	35.3	26.2	24.5	21.9	23.4	31.1	19.1	21.1	27.8	29.5	19.8	19.0	29.7	32.8
	Heptacosane	0.0	32.7	49.6	59.7	0.0	0.0	0.0	0.0	0.0	11.0	0.0	0.0	0.0	0.0
Percentage of M+2	Tricosene	0.0	0.0	0.0	0.0	8.5	8.8	0.0	0.0	0.0	12.0	0.0	9.7	0.0	0.0
	Tricosane	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	3 Me C23	0.0	0.0	2.9	0.0	0.0	5.5	0.0	0.0	0.0	0.0	0.0	8.4	0.0	6.1
	Pentacosene	13.9	4.1	3.1	2.7	4.1	3.2	4.3	3.4	3.1	2.8	4.3	3.8	3.9	1.7
	Pentacosane	0.0	0.0	0.0	0.0	0.0	0.0	13.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	3 Me C25	5.7	3.1	1.8	2.9	1.7	4.5	2.1	8.9	5.8	5.4	0.6	4.3	4.9	4.9
	Heptacosene	6.8	5.5	6.8	3.6	4.6	1.1	4.6	4.6	4.5	8.4	7.3	2.9	1.1	0.6
	Heptacosane	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Percentage of M+3	Tricosene	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Tricosane	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	3 Me C23	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Pentacosene	0.0	0.3	0.0	0.2	0.0	0.0	0.6	0.0	0.0	2.5	0.2	0.2	0.7	0.5
	Pentacosane	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	3 Me C25	1.1	1.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Heptacosene	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.2	0.0	0.0	0.0	0.0	0.0
	Heptacosane	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

0
0-20
20-40
40-60
60-80
80-100
100+

Figure C.25: Data sheet showing page four of the raw control data for the DL-valine d_8 experiment and *Formica lemani*.

DL-Valine d_8		*Formica lemani*											
		Val 1	Val 2	Val 3	Val 4	Val 5	Val 6	Val 7	Val 8	Val 9	Val 10	Val 11	Val 12
Abundance of M	Tricosene	4385	3718	2368	6123	5741	4395	7888	3764	9428	6147	16736	2773
	Tricosane	1435	736	733	1267	1033	1807	1244	1286	4447	994	7549	752
	3 Me C23	2762	4983	2360	4102	4127	4822	7116	4300	12430	10063	22792	3955
	Pentacosene	86112	92168	78664	129104	69528	89560	94248	93576	237760	116448	717952	61752
	Pentacosane	5861	1925	2981	4105	2192	9037	2894	3438	5147	2192	12401	1484
	3 Me C25	20704	24192	19224	25824	12456	25424	25312	21472	52976	25352	125040	15569
	Heptacosene	28800	24560	22368	40304	18880	33216	26872	28888	69464	24552	145600	21160
	Heptacosane	1207	258	528	277	466	2010	749	395	1373	660	4508	336
Abundance of M+1	Tricosene		166	901	1034	657	430	1383	266	4281	2101	5223	195
	Tricosane	295	488		436	463	629				159	1898	168
	3 Me C23	471	400	607	909	1133	1434	1606	1011	2112	2113	6488	986
	Pentacosene	23976	25664	21648	34000	16035	28624	23944	26960	51088	24928	193728	13988
	Pentacosane	3399			1095	328	2066	685	344	1502	463	3548	533
	3 Me C25	3687	3840	5810	7956	4479	7669	7019	6806	14856	7570	38184	5816
	Heptacosene	7286	6313	4624	12644	8711	8347	8143	10050	21088	9582	47464	4676
	Heptacosane	332					324					854	
Abundance of M+2	Tricosene			208	375			569		807		445	
	Tricosane												
	3 Me C23								160	1300			
	Pentacosene	1603	3235	3487	3990	1523	2687	2514	2331	6031	3551	17192	2813
	Pentacosane	3687										1631	
	3 Me C25		791	1573	1285	1201	547	1458	955	1062	1039	5628	826
	Heptacosene	1404	748	2102	2397	538	2816	1807	984	2559	1106	5321	756
	Heptacosane												
Abundance of M+3	Tricosene												
	Tricosane												
	3 Me C23												
	Pentacosene	285		189	767		496	278		886	150	2139	
	Pentacosane												
	3 Me C25				165	191		503	562	295	718	2270	623
	Heptacosene				171						282		
	Heptacosane												

Figure C.26: Data sheet showing page one of the raw substrate data for the DL-valine d_8 experiment and *Formica lemani*.

DL-Valine d_8		*Formica lemani*												
		Val 13	Val 14	Val 15	Val 16	Val 17	Val 18	Val 19	Val 20	Val 21	Val 22	Val 23	Val 24	Val 25
Abundance of M	Tricosene	14774	5314	5062	4452	5130	4627		3926	4411		2271	1052	
	Tricosane	4626	4522	794	4718	1784	1291		634	545		864	614	
	3 Me C23	14049	7534	6075	4562	4610	8355		8514	5107		3475	1492	
	Pentacosene	400064	207232	111504	115256	81600	155648		118448	101152		34752	34528	
	Pentacosane	7501	9412	6915	7973	2881	3651		2921	1325		1698	3220	
	3 Me C25	72400	50528	27144	29832	16752	34296		26408	24144		11822	12119	
	Heptacosene	75912	52576	794	38688	16840	51624		35592	29344		14163	12941	
	Heptacosane	1498	4125	1120	3390	205	2983		714	880		222	1752	
Abundance of M+1	Tricosene	4064	2504	937	188	707	708		823	1258		728	482	
	Tricosane	1865	1739	459	971	355	942			247			256	
	3 Me C23	5194	541	1287	1589	1598	1054		1050	922		1899		
	Pentacosene	99416	53392	18424	30864	17600	38400		31552	24776		9886	8635	
	Pentacosane	2334	3740	813		815	166		555	447			847	
	3 Me C25	14440	11538	4553	7226	5476	10612		9009	5162		2978	3506	
	Heptacosene	22656	14003	459	9590	4957	13831		8834	7759		3579	3965	
	Heptacosane	501	381		1029				361				449	
Abundance of M+2	Tricosene	1078	911						371					
	Tricosane													
	3 Me C23	1314				369			238					
	Pentacosene	14581	6954	4581	4274	2169	5242		5134	3246		2787	731	
	Pentacosane		1359	380			1995							
	3 Me C25	3537	951	1133	329				1051	773		389	1118	
	Heptacosene	3468	1369		1897	2292	1912		2303	1553		596	326	
	Heptacosane													
Abundance of M+3	Tricosene													
	Tricosane													
	3 Me C23								167					
	Pentacosene		225	418			403		200					
	Pentacosane													
	3 Me C25	1779							876			460		
	Heptacosene				443				257	346				
	Heptacosane													

Figure C.27: Data sheet showing page two of the raw substrate data for the DL-valine d_8 experiment and *Formica lemani*.

DL-Valine d_8		*Formica lemani*											
		Val 1	Val 2	Val 3	Val 4	Val 5	Val 6	Val 7	Val 8	Val 9	Val 10	Val 11	Val 12
Percentage of M+1	Tricosene	0.0	4.5	38.0	16.9	11.4	9.8	17.5	7.1	45.4	34.2	31.2	7.0
	Tricosane	20.6	66.3	0.0	34.4	44.8	34.8	0.0	0.0	0.0	16.0	25.1	22.3
	3 Me C23	17.1	8.0	25.7	22.2	27.5	29.7	22.6	23.5	17.0	21.0	28.5	24.9
	Pentacosene	27.8	27.8	27.5	26.3	23.1	32.0	25.4	28.8	21.5	21.4	27.0	22.7
	Pentacosane	58.0	0.0	0.0	26.7	15.0	22.9	23.7	10.0	29.2	21.1	28.6	35.9
	3 Me C25	17.8	15.9	30.2	30.8	36.0	30.2	27.7	31.7	28.0	29.9	30.5	37.4
	Heptacosene	25.3	25.7	20.7	31.4	46.1	25.1	30.3	34.8	30.4	39.0	32.6	22.1
	Heptacosane	27.5	0.0	0.0	0.0	0.0	16.1	0.0	0.0	0.0	0.0	18.9	0.0
Percentage of M+2	Tricosene	0.0	0.0	8.8	6.1	0.0	0.0	7.2	0.0	8.6	0.0	2.7	0.0
	Tricosane	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	3 Me C23	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.7	10.5	0.0	0.0	0.0
	Pentacosene	1.9	3.5	4.4	3.1	2.2	3.0	2.7	2.5	2.5	3.0	2.4	4.6
	Pentacosane	62.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	13.2	0.0
	3 Me C25	0.0	3.3	8.2	5.0	9.6	2.2	5.8	4.4	2.0	4.1	4.5	5.3
	Heptacosene	4.9	3.0	9.4	5.9	2.8	8.5	6.7	3.4	3.7	4.5	3.7	3.6
	Heptacosane	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Percentage of M+3	Tricosene	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Tricosane	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	3 Me C23	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Pentacosene	0.3	0.0	0.2	0.6	0.0	0.6	0.3	0.0	0.4	0.1	0.3	0.0
	Pentacosane	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	3 Me C25	0.0	0.0	0.0	0.6	1.5	0.0	2.0	2.6	0.6	2.8	1.8	4.0
	Heptacosene	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	1.1	0.0	0.0
	Heptacosane	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

0
0-20
20-40
40-60
60-80
80-100
100+

Figure C.28: Data sheet showing page three of the raw substrate data for the DL-valine d_8 experiment and *Formica lemani*.

DL-Valine d_8		*Formica lemani*												
		Val 13	Val 14	Val 15	Val 16	Val 17	Val 18	Val 19	Val 20	Val 21	Val 22	Val 23	Val 24	Val 25
Percentage of M+1	Tricosene	27.5	47.1	18.5	4.2	13.8	15.3		21.0	28.5		32.1	45.8	
	Tricosane	40.3	38.5	57.8	20.6	19.9	73.0		0.0	45.3		0.0	41.7	
	3 Me C23	37.0	7.2	21.2	34.8	34.7	12.6		12.3	18.1		54.6	0.0	
	Pentacosene	24.9	25.8	16.5	26.8	21.6	24.7		26.6	24.5		28.4	25.0	
	Pentacosane	31.1	39.7	11.8	0.0	28.3	4.5		19.0	33.7		0.0	26.3	
	3 Me C25	19.9	22.8	16.8	24.2	32.7	30.9		34.1	21.4		25.2	28.9	
	Heptacosene	29.8	26.6	57.8	24.8	29.4	26.8		24.8	26.4		25.3	30.6	
	Heptacosane	33.4	9.2	0.0	30.4	0.0	0.0		50.6	0.0		0.0	25.6	
Percentage of M+2	Tricosene	7.3	17.1	0.0	0.0	0.0	0.0		9.4	0.0		0.0	0.0	
	Tricosane	0.0	0.0	0.0	0.0	0.0	0.0		0.0	0.0		0.0	0.0	
	3 Me C23	9.4	0.0	0.0	0.0	8.0	0.0		2.8	0.0		0.0	0.0	
	Pentacosene	3.6	3.4	4.1	3.7	2.7	3.4		4.3	3.2		8.0	2.1	
	Pentacosane	0.0	14.4	5.5	0.0	0.0	54.6		0.0	0.0		0.0	0.0	
	3 Me C25	4.9	1.9	4.2	1.1	0.0	0.0		4.0	3.2		3.3	9.2	
	Heptacosene	4.6	2.6	0.0	4.9	13.6	3.7		6.5	5.3		4.2	2.5	
	Heptacosane	0.0	0.0	0.0	0.0	0.0	0.0		0.0	0.0		0.0	0.0	
Percentage of M+3	Tricosene	0.0	0.0	0.0	0.0	0.0	0.0		0.0	0.0		0.0	0.0	
	Tricosane	0.0	0.0	0.0	0.0	0.0	0.0		0.0	0.0		0.0	0.0	
	3 Me C23	0.0	0.0	0.0	0.0	0.0	0.0		2.0	0.0		0.0	0.0	
	Pentacosene	0.0	0.1	0.4	0.0	0.0	0.3		0.2	0.0		0.0	0.0	
	Pentacosane	0.0	0.0	0.0	0.0	0.0	0.0		0.0	0.0		0.0	0.0	
	3 Me C25	2.5	0.0	0.0	0.0	0.0	0.0		3.3	0.0		3.9	0.0	
	Heptacosene	0.0	0.0	0.0	1.1	0.0	0.0		0.7	1.2		0.0	0.0	
	Heptacosane	0.0	0.0	0.0	0.0	0.0	0.0		0.0	0.0		0.0	0.0	

0
0-20
20-40
40-60
60-80
80-100
100+

Figure C.29: Data sheet showing page four of the raw substrate data for the DL-valine d_8 experiment and *Formica lemani*.

DL-Valine (Negative Control)		*Myrmica sabuleti*													
		Con 1	Con 2	Con 3	Con 4	Con 5	Con 6	Con 7	Con 8	Con 9	Con 10	Con 11	Con 12	Con 13	Con 14
Abundance of M	Tricosane	5042	1876	28888	6036	3350	2855	7191	8627	2909	4095	3875	8893	12642	6906
	5 Me C23	12714	9477	96032	16752	9275	17008	19920	14326	17064	16143	16520	22184	24472	14862
	Pentacosene	95568	47688	720704	67752	66696	82608	74608	102848	71256	77992	80584	121096	129512	90824
	Pentacosane	2935	1383	9394	2590	2233	2408	3302	3746	4215	722	4451	1525	5855	4457
	11 13 Me C25	33008	20640	101648	14108	20848	33048	31472	33872	28544	22208	2611	44248	47528	32608
	5 Me C25	99584	54608	564480	85432	66528	66200	91152	136960	82344	76928	94936	133760	108592	92424
	Heptacosene	13008	5584	58008	13466	9588	21424	14851	17736	14331	14619	17008	22856	25696	11784
	5 Me C27	31792	20200	145920	31680	22616	22480	29840	38656	27104	30016	27888	46312	31776	30408
Abundance of M+1	Tricosane	1711	542	7868	1458	2351	1622	2270	2147	519	1748	1284	1108	4133	1858
	5 Me C23	1481	581	17696	3619	2771	3379	4116	3303	2796	2722	1330	5540	4071	5965
	Pentacosene	20392	10868	185600	15951	11691	20536	21544	24536	16888	21840	20320	27816	37640	23360
	Pentacosane	568	1175	3692	2208		454	1369	1512	225		1261	501	2297	1138
	11 13 Me C25	16688	9362	51792	7192	7529	17032	13752	21328	16185	12021	2277	21304	27856	22152
	5 Me C25	21408	11820	130456	21000	11008	15763	19416	33288	17960	17360	19584	27928	22832	19280
	Heptacosene	4198	2589	19488	4496	1748	5060	4747	4395	3991	5522	6155	3912	5843	3722
	5 Me C27	6604	3778	29504	6689	5315	6633	7130	10241	10217	7321	6311	9692	7736	6826
Abundance of M+2	Tricosane	217		1434			411							737	203
	5 Me C23			2967	589	170	873		574				540	585	
	Pentacosene	2100	1176	26120	2666	1034	3107	2608		2598	3383	1994	2869	2854	3744
	Pentacosane			260								822			
	11 13 Me C25	1159	1991	11189	988	1655	1817	3328	2603	2430	2996		1650	3316	2786
	5 Me C25	855	1582	9389	1656	1748	918	2084	1522	3089	1845	2055	2129	3592	2158
	Heptacosene	438	1034	2816	273		826	525	435		1270	791	1064	1341	159
	5 Me C27	649	939	3044	1140	954	733	425	1372	2316	909	1152	1244	1100	437
Abundance of M+3	Tricosane														
	5 Me C23												158		
	Pentacosene		589	1613		411				295			297		190
	Pentacosane														
	11 13 Me C25			723											
	5 Me C25			962			912	943		429				599	
	Heptacosene	419		308											
	5 Me C27			393			153		289		408				202

Figure C.30: Data sheet showing page one of the raw control data for the DL-valine d_8 experiment and *Myrmica sabuleti*.

DL-Valine (Negative Control)		*Myrmica sabuleti*												
		Con 15	Con 16	Con 17	Con 18	Con 19	Con 20	Con 21	Con 22	Con 23	Con 24	Con 25	Con 26	Con 27
Abundance of M	Tricosane	9499	16704	9480	7780	7007	5024	5122	28328	9987	27232	10519	15667	7178
	5 Me C23	33888	66584	29640	25088	18200	23128	17384	92896	20736	53968	33808	60904	23184
	Pentacosene	168576	241600	117136	103944	90280	99616	87792	500352	96904	289344	175808	332800	126056
	Pentacosane	4140	6769	2923	4130	2980	2891	1935	13201	1459	5707	6245	7601	2851
	11 13 Me C25	58784	77200	40536	37048	28320	27552	34720	91200	27544	70304	53768	88688	41288
	5 Me C25	152960	193792	131392	100320	108320	120824	105096	434432	92152	247104	132608	268288	129184
	Heptacosene	31272	38384	20040	15755	12644	21624	11667	67968	4468	45448	30744	58488	14601
	5 Me C27	47488	67704	36544	33072	29568	31096	28032	145728	31176	69528	41720	76224	36056
Abundance of M+1	Tricosane	4056	2552	3185	2893	2480	2345	2703	7541	4444	5633	1923	8832	3283
	5 Me C23	4883	13874	2708	4557	3508	8101	2616	14123	1555	9346	9303	9006	4849
	Pentacosene	46760	65280	33016	23560	21744	27864	27752	119976	23816	68248	47144	75568	34312
	Pentacosane	1518	1566		670	402	854		4254		1272	957	2750	262
	11 13 Me C25	27976	42392	21464	19784	18448	14098	14281	50880	21376	41120	18872	47248	20024
	5 Me C25	30792	33928	26208	28032	22120	22912	22144	92016	20000	56112	30360	67032	29952
	Heptacosene	7008	10019	5763	3981	4809	4776	1941	14392	3576	12844	6403	17424	3589
	5 Me C27	12435	16888	10677	7748	8454	7907	5458	36600	6138	15831	8650	15807	11925
Abundance of M+2	Tricosane		782		698		572		880		1024		2330	
	5 Me C23	1117	2909	707			338	539	1320	512	534	849	1718	294
	Pentacosene	5826	9044	3967	3921	2241	4334	6505	14658	2542	5207	5102	8508	4911
	Pentacosane						289							185
	11 13 Me C25	2705	3289	1766	2480	1729	1482	2600	5607	1262	5688	2911	3229	4169
	5 Me C25	3977	4477	1531	2790	4922	2219	3038	11281	1751	3394	3634	4059	3989
	Heptacosene	1754	2246	1519	227	687	1154	635	1077	923	1638		1787	378
	5 Me C27	1258	2005	568		625	2102	1284	4919		1592	1189	3608	
Abundance of M+3	Tricosane													
	5 Me C23													
	Pentacosene		629	865		503	931		652	972		203		
	Pentacosane													
	11 13 Me C25								665	710				
	5 Me C25	268	308				1597		2231			163		
	Heptacosene	167											710	
	5 Me C27			481			453	564	293					

Figure C.31: Data sheet showing page two of the raw control data for the DL-valine d_8 experiment and *Myrmica sabuleti*.

DL-Valine (Negative Control)		*Myrmica sabuleti*													
		Con 1	Con 2	Con 3	Con 4	Con 5	Con 6	Con 7	Con 8	Con 9	Con 10	Con 11	Con 12	Con 13	Con 14
Percentage of M+1	Tricosane	33.9	28.9	27.2	24.2	70.2	56.8	31.6	24.9	17.8	42.7	33.1	12.5	32.7	26.9
	5 Me C23	11.6	6.1	18.4	21.6	29.9	19.9	20.7	23.1	16.4	16.9	8.1	25.0	16.6	40.1
	Pentacosene	21.3	22.8	25.8	23.5	17.5	24.9	28.9	23.9	23.7	28.0	25.2	23.0	29.1	25.7
	Pentacosane	19.4	85.0	39.3	85.3	0.0	18.9	41.5	40.4	5.3	0.0	28.3	32.9	39.2	25.5
	11 13 Me C25	50.6	45.4	51.0	51.0	36.1	51.5	43.7	63.0	56.7	54.1	87.2	48.1	58.6	67.9
	5 Me C25	21.5	21.6	23.1	24.6	16.5	23.8	21.3	24.3	21.8	22.6	20.6	20.9	21.0	20.9
	Heptacosene	32.3	46.4	33.6	33.4	18.2	23.6	32.0	24.8	27.8	37.8	36.2	17.1	22.7	31.6
	5 Me C27	20.8	18.7	20.2	21.1	23.5	29.5	23.9	26.5	37.7	24.4	22.6	20.9	24.3	22.4
Percentage of M+2	Tricosane	4.3	0.0	5.0	0.0	0.0	14.4	0.0	0.0	0.0	0.0	0.0	0.0	5.8	2.9
	5 Me C23	0.0	0.0	3.1	3.5	1.8	5.1	0.0	4.0	0.0	0.0	0.0	2.4	2.4	0.0
	Pentacosene	2.2	2.5	3.6	3.9	1.6	3.8	3.5	0.0	3.6	4.3	2.5	2.4	2.2	4.1
	Pentacosane	0.0	0.0	2.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	18.5	0.0	0.0	0.0
	11 13 Me C25	3.5	9.6	11.0	7.0	7.9	5.5	10.6	7.7	8.5	13.5	0.0	3.7	7.0	8.5
	5 Me C25	0.9	2.9	1.7	1.9	2.6	1.4	2.3	1.1	3.8	2.4	2.2	1.6	3.3	2.3
	Heptacosene	3.4	18.5	4.9	2.0	0.0	3.9	3.5	2.5	0.0	8.7	4.7	4.7	5.2	1.3
	5 Me C27	2.0	4.6	2.1	3.6	4.2	3.3	1.4	3.5	8.5	3.0	4.1	2.7	3.5	1.4
Percentage of M+3	Tricosane	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	5 Me C23	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0
	Pentacosene	0.0	1.2	0.2	0.0	0.6	0.0	0.0	0.0	0.4	0.0	0.0	0.2	0.0	0.2
	Pentacosane	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	11 13 Me C25	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	5 Me C25	0.0	0.0	0.2	0.0	0.0	1.4	1.0	0.0	0.5	0.0	0.0	0.0	0.6	0.0
	Heptacosene	3.2	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	5 Me C27	0.0	0.0	0.3	0.0	0.0	0.7	0.0	0.7	0.0	1.4	0.0	0.0	0.0	0.7

0
0-20
20-40
40-60
60-80
80-100
100+

Figure C.32: Data sheet showing page three of the raw control data for the DL-valine d_8 experiment and *Myrmica sabuleti*.

DL-Valine (Negative Control)		*Myrmica sabuleti*													
		Con 14	Con 15	Con 16	Con 17	Con 18	Con 19	Con 20	Con 21	Con 22	Con 23	Con 24	Con 25	Con 26	Con 27
Percentage of M+1	Tricosane	26.9	42.7	15.3	33.6	37.2	35.4	46.7	52.8	26.6	44.5	20.7	18.3	56.4	45.7
	5 Me C23	40.1	14.4	20.8	9.1	18.2	19.3	35.0	15.0	15.2	7.5	17.3	27.5	14.8	20.9
	Pentacosene	25.7	27.7	27.0	28.2	22.7	24.1	28.0	31.6	24.0	24.6	23.6	26.8	22.7	27.2
	Pentacosane	25.5	36.7	23.1	0.0	16.2	13.5	29.5	0.0	32.2	0.0	22.3	15.3	36.2	9.2
	11 13 Me C25	67.9	47.6	54.9	53.0	53.4	65.1	51.2	41.1	55.8	77.6	58.5	35.1	53.3	48.5
	5 Me C25	20.9	20.1	17.5	19.9	27.9	20.4	19.0	21.1	21.2	21.7	22.7	22.9	25.0	23.2
	Heptacosene	31.6	22.4	26.1	28.8	25.3	38.0	22.1	16.6	21.2	80.0	28.3	20.8	29.8	24.6
	5 Me C27	22.4	26.2	24.9	29.2	23.4	28.6	25.4	19.5	25.1	19.7	22.8	20.7	20.7	33.1
Percentage of M+2	Tricosane	2.9	0.0	4.7	0.0	9.0	0.0	11.4	0.0	3.1	0.0	3.8	0.0	14.9	0.0
	5 Me C23	0.0	3.3	4.4	2.4	0.0	0.0	1.5	3.1	1.4	2.5	1.0	2.5	2.8	1.3
	Pentacosene	4.1	3.5	3.7	3.4	3.8	2.5	4.4	7.4	2.9	2.6	1.8	2.9	2.6	3.9
	Pentacosane	0.0	0.0	0.0	0.0	0.0	0.0	10.0	0.0	0.0	0.0	0.0	0.0	0.0	6.5
	11 13 Me C25	8.5	4.6	4.3	4.4	6.7	6.1	5.4	7.5	6.1	4.6	8.1	5.4	3.6	10.1
	5 Me C25	2.3	2.6	2.3	1.2	2.8	4.5	1.8	2.9	2.6	1.9	1.4	2.7	1.5	3.1
	Heptacosene	1.3	5.6	5.9	7.6	1.4	5.4	5.3	5.4	1.6	20.7	3.6	0.0	3.1	2.6
	5 Me C27	1.4	2.6	3.0	1.6	0.0	2.1	6.8	4.6	3.4	0.0	2.3	2.8	4.7	0.0
Percentage of M+3	Tricosane	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	5 Me C23	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Pentacosene	0.2	0.0	0.3	0.7	0.0	0.6	0.9	0.0	0.1	1.0	0.0	0.1	0.0	0.0
	Pentacosane	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	11 13 Me C25	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	2.6	0.0	0.0	0.0	0.0
	5 Me C25	0.0	0.2	0.2	0.0	0.0	0.0	1.3	0.0	0.5	0.0	0.0	0.1	0.0	0.0
	Heptacosene	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.2	0.0
	5 Me C27	0.7	0.0	0.0	1.3	0.0	0.0	1.5	2.0	0.2	0.0	0.0	0.0	0.0	0.0

0
0-20
20-40
40-60
60-80
80-100
100+

Figure C.33: Data sheet showing page four of the raw control data for the DL-valine d_8 experiment and *Myrmica sabuleti*.

DL-Valine d_8		*Myrmica sabuleti*																							
		Val 1	Val 2	Val 3	Val 4	Val 5	Val 6	Val 7	Val 8	Val 9	Val 10	Val 11	Val 12	Val 13	Val 14	Val 15	Val 16	Val 17	Val 18	Val 19	Val 20	Val 21	Val 22	Val 23	Val 24	Val 25
Abundance of M	Tricosane	5241	18320	4102	3682	8133	4546	6909	6047	6562	8164	6836		6968	3828	3680	2703	5995	3653	20440	10755	3077	2709	3372	7887	12319
	5 Me C23	7305	19600	10915	21080	7128	8473	6101	8469	6803	13266	9985		16122	10197	7567	4926	7793	6211	28576	13525	4661	5817	7396	16257	14448
	Pentacosene	68088	215488	110584	173120	70944	67264	65096	56512	53368	109448	89024		128576	84248	4888	50264	78600	50648	232832	99792	37968	75776	70024	99672	157952
	Pentacosane	915	4989	2279	2845	2978	2972	2873	1715	2137	2314	3126		3253	2172	1844	2197	2652	1771	6659	2333	2566	1073	761	3921	4662
	11 13 Me C25	28592	51648	42192	46168	22608	26184	17528	19112	18376	31200	23584		32560	33264	15173	12756	20256	19424	60776	38016	60008	24256	12487	24968	38776
	5 Me C25	56328	119712	71088	93768	47544	45152	37832	40800	29664	64992	52224		77128	50336	17272	23648	14025	26176	112272	71256	9084	36456	28648	42968	107416
	Heptacosene	13321	27824	21624	28296	13663	9464	10118	9695	14487	19480	17736		19168	12784	8927	11293	17600	10180	28704	14061	7870	11932	10862	17656	20112
	5 Me C27	13893	32648	8296	27312	11220	13322	6573	3603	4141	20352	4220		17576	16528	4101	1631	5581	1616	36936	23104	1915	6239	3767	3419	18880
Abundance of M+1	Tricosane	2005	2538	1361	1783	2390	3144	3162	2185	1260	2857	2307		1222	1671	574	1916	1251	1104	5500	3082	2333	1383	862	2710	2854
	5 Me C23	1399	4151	1945	6863	1507	798	1552	1867	2297	4192	3289		3197	2027	1681	1483	1390	564	4361	3695	1324	1145		2179	9436
	Pentacosene	25192	53000	30592	51568	19832	23728	13754	13515	14198	28840	24656		33448	26856	4026	14246	23536	12896	64448	24600	13960	15664	10316	38248	38640
	Pentacosane	759	780	1306	1250	563	802	1459	409	437	1264	687		682		674	1668	429	967	1270	1097				447	1121
	11 13 Me C25	17216	34560	19344	21352	11262	15593	9647	6615	9560	19792	12534		19144	17528	9759	10129	11426	8741	30040	14399	2529	9394	12446	16416	13263
	5 Me C25	9158	23440	15683	23896	9233	8795	6784	10409	10661	16025	13521		19280	11356	3238	7499	5248	9263	30864	16808	3623	12002	8529	11724	20040
	Heptacosene	4797	8659	8279	7433	4019	2844	2368	2630	6194	5892	4103		5247	5046	3179	2508	3548	2424	11765	6137	937	4425	5276	6355	4549
	5 Me C27	3776	9283	2538	5661	5259	3236	2866	1935	2152	5857	2626		5587	4592	2230	788	2570	1544	10230	4292	704	2526	1667	5090	5756
Abundance of M+2	Tricosane		1465		252		534		289		728					647				307		310				834
	5 Me C23	1439	4905	1090	2479	2833		1249	520	1122	613	1919		1047	776	2041	570	1327	1023	1333	3100	529	2062	2969		4741
	Pentacosene	1612	4896	3380	4244	3151	1962	3229	3112	2752	4427	3948		4325	2734	3463	1979	2006	3428	11184	4342	2374	334		4693	5551
	Pentacosane									251		217					1852					882				245
	11 13 Me C25	1906	4341	2743	2490	916	1313	1474	1780	2562	2879	1935			3607	1253		4504	1744	4276	2080		717	2023	1852	2056
	5 Me C25	2811	3946	7520	6827	2213	2003	2733	4496	4791	5682	6781		3639	2292	16245	5171	22056	8838	5928	6756	4834	6097	3070	26608	8097
	Heptacosene	693	1782	507	465	653	603	886	949	706	1165	1111		787	166	1733	1216	257	436	3090	1069		642		1765	634
	5 Me C27	3263	4935	13389	6349	2769	3292	4856	4393	4069	3466	11069		3329	2786	9035	10959	10271	6359	2591	2490	3853	2987	1770	15325	5989
Abundance of M+3	Tricosane																									
	5 Me C23	731	2430	2083	1492	1921		3123	1178	627	342	2012		4199	488	2076		1065	3665	1788	3077	283	2427			4605
	Pentacosene			803	176			711	414			565		294	332	1683				3454	175					
	Pentacosane																									
	11 13 Me C25		2112	2590	4261	2609	1979	947	1189	1713	1459	2483				1041		1519	1352	1885			1871	1516	2868	1272
	5 Me C25	1761	7178	5798	14411	4293	901	5443	6224	6381	7011	9288		3347	1226	17104	11160	37912	8572	6418	5043	11792	6683	1835	43976	5395
	Heptacosene															735										
	5 Me C27	2858	4012	13373	6398	2446	3240	6207	11566	9529	6369	22152		4010	3372	15906	10095	17688	12924	3862	5626	3075	2681	5249	31816	12096

Figure C.34: Data sheet showing page one of the raw substrate data for the DL-valine d_8 experiment and *Myrmica sabuleti*.

DL-Valine d_8		*Myrmica sabuleti*																							
		Val 1	Val 2	Val 3	Val 4	Val 5	Val 6	Val 7	Val 8	Val 9	Val 10	Val 11	Val 12	Val 13	Val 14	Val 15	Val 16	Val 17	Val 18	Val 19	Val 20	Val 21	Val 22	Val 23	Val 24	Val 25
Percentage of M+1	Tricosane	38.3	13.9	33.2	48.4	29.4	69.2	45.8	36.1	19.2	35.0	33.7		17.5	43.7	15.6	70.9	20.9	30.2	26.9	28.7	75.8	51.1	25.6	34.4	23.2
	5 Me C23	19.2	21.2	17.8	32.6	21.1	9.4	25.4	22.0	33.8	31.6	32.9		19.8	19.9	22.2	30.1	17.8	9.1	15.3	27.3	28.4	19.7	0.0	13.4	65.3
	Pentacosene	37.0	24.6	27.7	29.8	28.0	35.3	21.1	23.9	26.6	26.4	27.7		26.0	31.9	82.4	28.3	29.9	25.5	27.7	24.7	36.8	20.7	14.7	38.4	24.5
	Pentacosane	83.0	15.6	57.3	43.9	18.9	27.0	50.8	23.8	20.4	54.6	22.0		21.0	0.0	36.6	75.9	16.2	54.6	19.1	47.0	0.0	0.0	0.0	11.4	24.0
	11 13 Me C25	60.2	66.9	45.8	46.2	49.8	59.6	55.0	34.6	52.0	63.4	53.1		58.8	52.7	64.3	79.4	56.4	45.0	49.4	37.9	4.2	38.7	99.7	65.7	34.2
	5 Me C25	16.3	19.6	22.1	25.5	19.4	19.5	17.9	25.5	35.9	24.7	25.9		25.0	22.6	18.7	31.7	37.4	35.4	27.5	23.6	39.9	32.9	29.8	27.3	18.7
	Heptacosene	36.0	31.1	38.3	26.3	29.4	30.1	23.4	27.1	42.8	30.2	23.1		27.4	39.5	35.6	22.2	20.2	23.8	41.0	43.6	11.9	37.1	48.6	36.0	22.6
	5 Me C27	27.2	28.4	30.6	20.7	46.9	24.3	43.6	53.7	52.0	28.8	62.2		31.8	27.8	54.4	48.3	46.0	95.5	27.7	18.6	36.8	40.5	44.3	148.9	30.5
Percentage of M+2	Tricosane	0.0	8.0	0.0	6.8	0.0	11.7	0.0	4.8	0.0	8.9	0.0		0.0	0.0	17.6	0.0	0.0	0.0	1.5	0.0	10.1	0.0	0.0	0.0	6.8
	5 Me C23	19.7	25.0	10.0	11.8	39.7	0.0	20.5	6.1	16.5	4.6	19.2		6.5	7.6	27.0	11.6	17.0	16.5	4.7	22.9	11.3	35.4	40.1	0.0	32.8
	Pentacosene	2.4	2.3	3.1	2.5	4.4	2.9	5.0	5.5	5.2	4.0	4.4		3.4	3.2	70.8	3.9	2.6	6.8	4.8	4.4	6.3	0.4	0.0	4.7	3.5
	Pentacosane	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	11.7	0.0	6.9		0.0	0.0	0.0	84.3	0.0	0.0	0.0	0.0	34.4	0.0	0.0	0.0	5.3
	11 13 Me C25	6.7	8.4	6.5	5.4	4.1	5.0	8.4	9.3	13.9	9.2	8.2		0.0	10.8	8.3	0.0	22.2	9.0	7.0	5.5	0.0	3.0	16.2	7.4	5.3
	5 Me C25	5.0	3.3	10.6	7.3	4.7	4.4	7.2	11.0	16.2	8.7	13.0		4.7	4.6	94.1	21.9	157.3	33.8	5.3	9.5	53.2	16.7	10.7	61.9	7.5
	Heptacosene	5.2	6.4	2.3	1.6	4.8	6.4	8.8	9.8	4.9	6.0	6.3		4.1	1.3	19.4	10.8	1.5	4.3	10.8	7.6	0.0	5.4	0.0	10.0	3.2
	5 Me C27	23.5	15.1	161.4	23.2	24.7	24.7	73.9	121.9	98.3	17.0	262.3		18.9	16.9	220.3	671.9	184.0	393.5	7.0	10.8	201.2	47.9	47.0	448.2	31.7
Percentage of M+3	Tricosane	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	5 Me C23	10.0	12.4	19.1	7.1	27.0	0.0	51.2	13.9	9.2	2.6	20.2		26.0	4.8	27.4	0.0	13.7	59.0	6.3	22.8	6.1	41.7	0.0	0.0	31.9
	Pentacosene	0.0	0.0	0.7	0.1	0.0	0.0	1.1	0.7	0.0	0.0	0.6		0.2	0.4	34.4	0.0	0.0	0.0	1.5	0.2	0.0	0.0	0.0	0.0	0.0
	Pentacosane	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	11 13 Me C25	0.0	4.1	6.1	9.2	11.5	7.6	5.4	6.2	9.3	4.7	10.5		0.0	0.0	6.9	0.0	7.5	7.0	3.1	0.0	0.0	7.7	12.1	11.5	3.3
	5 Me C25	3.1	6.0	8.2	15.4	9.0	2.0	14.4	15.3	21.5	10.8	17.8		4.3	2.4	99.0	47.2	270.3	32.7	5.7	7.1	129.8	18.3	6.4	102.3	5.0
	Heptacosene	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.0	0.0	8.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	5 Me C27	20.6	12.3	161.2	23.4	21.8	24.3	94.4	321.0	230.1	31.3	524.9		22.8	20.4	387.9	618.9	316.9	799.8	10.5	24.4	160.6	43.0	139.3	930.6	64.1

0
0-20
20-40
40-60
60-80
80-100
100+

Figure C.35: Data sheet showing page two of the raw substrate data for the DL-valine d_8 experiment and *Myrmica sabuleti*.

DL-Valine (Negative Control)		*Formica lemani*									
		Con 1	Con 2	Con 3	Con 4	Con 5	Con 6	Con 7	Con 8	Con 9	Con 10
Abundance of M	Tricosene	1261	1197	8003	5292		2253	1434	3918	2738	3240
	Tricosane	23336	19880	79168	38456		31616	23896	75416	35112	36168
	3 Me C23	1303	1573	9156	4216		3640	5621	7865	8706	4086
	Pentacosene	4692	3907	28512	19456		9968	6139	17656	10814	12973
	Pentacosane	13080	10109	50112	26720		19832	12592	42592	19968	22784
	3 Me C25	224	352	1983	869		630	2300	1846	3357	876
	Heptacosene	1259	1209	8838	5261		2788	3353	5459	5165	3549
	Heptacosane	735	652	4406	3802		1555	721	3085	1529	1971
Abundance of M+1	Tricosene	284	304	1889	1218		579	279	882	684	724
	Tricosane	6427	5183	21072	9917		8581	6435	19488	9666	9702
	3 Me C23	357	433	2331	1167		947	1646	2284	2378	1127
	Pentacosene	1286	1039	7482	4696		2709	1548	4394	2845	3262
	Pentacosane	4041	3048	14793	7732		5691	3713	13124	6131	6803
	3 Me C25			570	269		157	684	553	1161	265
	Heptacosene	335	301	2320	1506		743	878	1478	1289	1003
	Heptacosane	209	201	1483	1112		495	239	1002	467	628
Abundance of M+2	Tricosene			214							
	Tricosane	832	687	2878	1387		1126	784	2591	1263	1287
	3 Me C23			339				199	291	263	170
	Pentacosene	189		1007	582		288	180	574	331	418
	Pentacosane	589	450	2036	1002		800	505	1911	919	953
	3 Me C25										
	Heptacosene			363	231				193	192	
	Heptacosane			265	157						
Abundance of M+3	Tricosene										
	Tricosane										
	3 Me C23										
	Pentacosene										
	Pentacosane										
	3 Me C25										
	Heptacosene										
	Heptacosane										

Figure C.36: Data sheet showing page one of the L-valine $^{13}C_5$,^{15}N experiment and *Formica lemani*.

DL-Valine (Negative Control)		*Formica lemani*									
		Con 1	Con 2	Con 3	Con 4	Con 5	Con 6	Con 7	Con 8	Con 9	Con 10
Percentage of M+1	Tricosene	22.5	25.4	23.6	23.0		25.7	19.5	22.5	25.0	22.3
	Tricosane	27.5	26.1	26.6	25.8		27.1	26.9	25.8	27.5	26.8
	3 Me C23	27.4	27.5	25.5	27.7		26.0	29.3	29.0	27.3	27.6
	Pentacosene	27.4	26.6	26.2	24.1		27.2	25.2	24.9	26.3	25.1
	Pentacosane	30.9	30.2	29.5	28.9		28.7	29.5	30.8	30.7	29.9
	3 Me C25	0.0	0.0	28.7	31.0		24.9	29.7	30.0	34.6	30.3
	Heptacosene	26.6	24.9	26.3	28.6		26.6	26.2	27.1	25.0	28.3
	Heptacosane	28.4	30.8	33.7	29.2		31.8	33.1	32.5	30.5	31.9
Percentage of M+2	Tricosene	0.0	0.0	2.7	0.0		0.0	0.0	0.0	0.0	0.0
	Tricosane	3.6	3.5	3.6	3.6		3.6	3.3	3.4	3.6	3.6
	3 Me C23	0.0	0.0	3.7	0.0		0.0	3.5	3.7	3.0	4.2
	Pentacosene	4.0	0.0	3.5	3.0		2.9	2.9	3.3	3.1	3.2
	Pentacosane	4.5	4.5	4.1	3.8		4.0	4.0	4.5	4.6	4.2
	3 Me C25	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0
	Heptacosene	0.0	0.0	4.1	4.4		0.0	0.0	3.5	3.7	0.0
	Heptacosane	0.0	0.0	6.0	4.1		0.0	0.0	0.0	0.0	0.0
Percentage of M+3	Tricosene	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0
	Tricosane	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0
	3 Me C23	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0
	Pentacosene	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0
	Pentacosane	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0
	3 Me C25	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0
	Heptacosene	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0
	Heptacosane	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0

0
0-20
20-40
40-60
60-80
80-100
100+

Figure C.37: Data sheet showing page two of the raw control data for the L-valine $^{13}C_5$,^{15}N experiment and *Formica lemani*.

DL-Valine (Negative Control)		*Myrmica sabuleti*															
		Con 1	Con 2	Con 3	Con 4	Con 5	Con 6	Con 7	Con 8	Con 9	Con 10	Con 11	Con 12	Con 13	Con 14	Con 15	Con 16
Abundance of M	Tricosane	1482	3548	11227	6284	1964			622	620	914	363	184				
	5 Me C23	7048	14344	36664	19232	10058			1980	3217	3678	1480	725				
	Pentacosene	22168	28392	71520	31424	21000			10930	14120	17160	6176	3809				
	Pentacosane	863	1111	3116	1874	669			209	278	308	168					
	5 Me C23	47120	53280	130504	63664	52440			17288	31040	28200	13338	8742				
	Heptacosene	3205	6041	14643	6561	3132			799	1011	1229	472	261				
	5 Me C27	8336	11000	27176	13056	9167			1599	2695	1502	1051	730				
Abundance of M+1	Tricosane	415	1055	2821	1514	507			159	150	231						
	5 Me C23	1454	2864	7535	3865	2039			470	616	774	254	168				
	Pentacosene	6055	7723	19512	8412	5916			3020	3657	4779	1562	1129				
	Pentacosane	240	324	869	554	188											
	5 Me C23	10707	11475	28776	13815	11643			3995	6797	5913	2855	1963				
	Heptacosene	990	1713	4230	1922	955			252	286	362						
	5 Me C27	2110	2689	6534	3224	2267			345	616	432	285	176				
Abundance of M+2	Tricosane			361	190												
	5 Me C23	199	293	834	397	228											
	Pentacosene	951	1012	2487	1133	767			386	423	596	199					
	Pentacosane																
	5 Me C23	1420	1239	3219	1590	1244			407	785	659	335	202				
	Heptacosene	178	281	672	242	180											
	5 Me C27	327	350	834	403	304											
Abundance of M+3	Tricosane																
	5 Me C23																
	Pentacosene			258													
	Pentacosane																
	5 Me C23	284	169	388	169												
	Heptacosene																
	5 Me C27																

Figure C.38: Data sheet showing page one of the raw control data for the L-valine $^{13}C_5$,^{15}N experiment and *Myrmica sabuleti*.

DL-Valine (Negative Control)		*Myrmica sabuleti*															
		Con 1	Con 2	Con 3	Con 4	Con 5	Con 6	Con 7	Con 8	Con 9	Con 10	Con 11	Con 12	Con 13	Con 14	Con 15	Con 16
Percentage of M+1	Tricosane	28.0	29.7	25.1	24.1	25.8			25.6	24.2	25.3	0.0	0.0				
	5 Me C23	20.6	20.0	20.6	20.1	20.3			23.7	19.1	21.0	17.2	23.2				
	Pentacosene	27.3	27.2	27.3	26.8	28.2			27.6	25.9	27.8	25.3	29.6				
	Pentacosane	27.8	29.2	27.9	29.6	28.1			0.0	0.0	0.0	0.0					
	5 Me C23	22.7	21.5	22.0	21.7	22.2			23.1	21.9	21.0	21.4	22.5				
	Heptacosene	30.9	28.4	28.9	29.3	30.5			31.5	28.3	29.5	0.0	0.0				
	5 Me C27	25.3	24.4	24.0	24.7	24.7			21.6	22.9	28.8	27.1	24.1				
Percentage of M+2	Tricosane	0.0	0.0	3.2	3.0	0.0			0.0	0.0	0.0	0.0	0.0				
	5 Me C23	2.8	2.0	2.3	2.1	2.3			0.0	0.0	0.0	0.0	0.0				
	Pentacosene	4.3	3.6	3.5	3.6	3.7			3.5	3.0	3.5	3.2	0.0				
	Pentacosane	0.0	0.0	0.0	0.0	0.0			0.0	0.0	0.0	0.0					
	5 Me C23	3.0	2.3	2.5	2.5	2.4			2.4	2.5	2.3	2.5	2.3				
	Heptacosene	5.6	4.7	4.6	3.7	5.7			0.0	0.0	0.0	0.0	0.0				
	5 Me C27	3.9	3.2	3.1	3.1	3.3			0.0	0.0	0.0	0.0	0.0				
Percentage of M+3	Tricosane	0.0	0.0	0.0	0.0	0.0			0.0	0.0	0.0	0.0	0.0				
	5 Me C23	0.0	0.0	0.0	0.0	0.0			0.0	0.0	0.0	0.0	0.0				
	Pentacosene	0.0	0.0	0.4	0.0	0.0			0.0	0.0	0.0	0.0	0.0				
	Pentacosane	0.0	0.0	0.0	0.0	0.0			0.0	0.0	0.0	0.0					
	5 Me C23	0.6	0.3	0.3	0.3	0.0			0.0	0.0	0.0	0.0	0.0				
	Heptacosene	0.0	0.0	0.0	0.0	0.0			0.0	0.0	0.0	0.0	0.0				
	5 Me C27	0.0	0.0	0.0	0.0	0.0			0.0	0.0	0.0	0.0	0.0				

0
0-20
20-40
40-60
80-100
100+

Figure C.39: Data sheet showing page two of the raw control data for the L-valine $^{13}C_5$,^{15}N experiment and *Myrmica sabuleti*.

L-Valine $^{13}C_5$, ^{15}N		*Myrmica sabuleti*											
		Val 1	Val 2	Val 3	Val 4	Val 5	Val 6	Val 7	Val 8	Val 9	Val 10	Val 11	Val 12
Abundance of M	Tricosane	272	156	112	209	228	204					200	
	5 Me C23	648	402	328	764	705	453	200	218	176	364	596	479
	Pentacosene	7030	3669	3140	6046	5274	3969	761	1740	1096	3712	4964	4092
	Pentacosane												
	5 Me C23	6493	3530	2715	4461	4579	4064	854	1376	1370	2995	4431	4096
	Heptacosene	486	292	223	600	432	280						313
	5 Me C27	423	165	195	449	280	262					292	267
Abundance of M+1	Tricosane												
	5 Me C23	257	188		330	288	192	200		176	169	264	626
	Pentacosene	2557	1391	1255	2342	2016	1487	368	559	530	1294	1613	1566
	Pentacosane												
	5 Me C23	2732	1487	1263	1991	1807	1418	442	566	645	1249	1904	1747
	Heptacosene	192			236	181							
	5 Me C27	205	199	195	254	395	196						238
Abundance of M+2	Tricosane	182			214	219	154						
	5 Me C23	647	470	366	897	755	376	200	179	176	353	603	936
	Pentacosene	3807	2072	1734	3147	2591	1590	513	857	850	1976	1981	2159
	Pentacosane												
	5 Me C23	5533	3198	2847	4281	4209	2861	1142	1309	1958	2565	3922	3727
	Heptacosene	222			321	153							157
	5 Me C27	523	232	195	574	468	304					282	402
Abundance of M+3	Tricosane												
	5 Me C23	828	597	572	1144	1121	535	243	213	292	573	827	1032
	Pentacosene	1956	1100	1140	1826	1223	812	290	541	528	1143	1096	1297
	Pentacosane												
	5 Me C23	8845	4833	4443	6618	6082	3945	1709	1940	3347	4207	5617	5603
	Heptacosene				160								
	5 Me C27	817	358	231	913	520	322					495	464
Abundance of M+4	Tricosane	189	172		254	174						103	
	5 Me C23	1075	721	648	1324	993	480	263	308	338	634	867	990
	Pentacosene	3335	1872	1843	2928	1961	1302	474	748	732	1730	1557	1902
	Pentacosane												
	5 Me C23	10246	5386	5303	7817	5883	3910	1873	2036	4026	4885	6367	6644
	Heptacosene	218			279	162							160
	5 Me C27	945	386	442	1078	604	266					450	649
Abundance of M+5	Tricosane												
	5 Me C23	1255	792	784	1443	1080	522	239	297	334	714	1008	841
	Pentacosene	1832	1025	1025	1685	999	751	239	557	481	1119	1062	1197
	Pentacosane												
	5 Me C23	12610	6503	6767	9435	6419	4519	2130	2814	4898	5610	7110	7908
	Heptacosene	159			172								
	5 Me C27	1363	353	445	1501	473	248					687	612

Figure C.40: Data sheet showing page one of the raw substrate data for the L-valine $^{13}C_5$,^{15}N experiment and *Myrmica sabuleti*.

L-Valine $^{13}C_5$, ^{15}N		*Myrmica sabuleti*											
		Val 1	Val 2	Val 3	Val 4	Val 5	Val 6	Val 7	Val 8	Val 9	Val 10	Val 11	Val 12
Abundance of M+6	Tricosane	179			162								
	5 Me C23	1096	616	668	1266	743	415	192	357	310	582	833	554
	Pentacosene	2326	1361	1385	2130	1232	825	286	634	573	1266	1251	1514
	Pentacosane												
	5 Me C23	11652	5310	5872	8111	4897	3291	1580	2499	4106	4651	5838	6928
	Heptacosene				206								
	5 Me C27	1150	330	430	1359	491	213					520	606
Abundance of M+7	Tricosane												
	5 Me C23	1155	569	569	1100	713	364		245	330	547	777	544
	Pentacosene	1325	762	728	1193	562	452		374	331	700	684	761
	Pentacosane												
	5 Me C23	11862	4877	5535	8383	4707	3340	1450	2389	4202	4567	5740	6763
	Heptacosene												
	5 Me C27	1300	282	507	1351	268						553	440
Abundance of M+8	Tricosane												
	5 Me C23	830	354	409	843	405	208		213	230	368	478	455
	Pentacosene	1384	677	749	1143	617	450		425	337	681	691	817
	Pentacosane												
	5 Me C23	8819	3341	3989	5640	2815	2021	868	1819	4898	3104	3873	4848
	Heptacosene												
	5 Me C27	911	226	382	1027	241						466	396
Abundance of M+9	Tricosane												
	5 Me C23	708	247	296	587	236	181		158		280	447	274
	Pentacosene	727	363	367	645	295	221		172		350	375	443
	Pentacosane												
	5 Me C23	7590	2551	3244	4730	2394	1630	671	1342	2967	2534	3339	3955
	Heptacosene												
	5 Me C27	940		274	956							389	263
Abundance of M+10	Tricosane												
	5 Me C23	366		193	350						178	207	
	Pentacosene	762	294	323	542	234	169		219		283	311	430
	Pentacosane												
	5 Me C23	4805	1601	1854	2803	1281	962	337	903	2405	1467	1974	2236
	Heptacosene												
	5 Me C27	640		214	573							225	189
Abundance of M+11	Tricosane												
	5 Me C23	248			217							155	
	Pentacosene	378	153		278							182	213
	Pentacosane												
	5 Me C23	3388	1032	1173	1932	858	687	218	771	1417	1003	1246	1565
	Heptacosene												
	5 Me C27	474			422							163	
Abundance of M+12	Tricosane												
	5 Me C23												
	Pentacosene	291			171							155	151
	Pentacosane												
	5 Me C23	1979	457	637	1083	376	331		352	986	493	636	780
	Heptacosene												
	5 Me C27	277			211								

Figure C.41: Data sheet showing page two of the raw substrate data for the L-valine $^{13}C_5$, ^{15}N experiment and *Myrmica sabuleti*.

L-Valine $^{13}C_5$, ^{15}N		*Myrmica sabuleti*											
		Val 1	Val 2	Val 3	Val 4	Val 5	Val 6	Val 7	Val 8	Val 9	Val 10	Val 11	Val 12
Abundance of M+13	Tricosane												
	5 Me C23												
	Pentacosene												
	Pentacosane												
	5 Me C23	1477	478	494	809	478	364		278	520	410	591	670
	Heptacosene												
	5 Me C27	205			176								
Abundance of M+14	Tricosane												
	5 Me C23												
	Pentacosene												
	Pentacosane												
	5 Me C23	980	402	400	683	499	377		220	341	354	531	592
	Heptacosene												
	5 Me C27	167											
Abundance of M+15	Tricosane												
	5 Me C23												
	Pentacosene												
	Pentacosane												
	5 Me C23	720	274	224	484	289	207		152	241	249	360	374
	Heptacosene												
	5 Me C27												
Percentage of M+1	Tricosane	0.0	0.0	0.0	0.0	0.0	0.0					0.0	
	5 Me C23	39.7	46.8	0.0	43.2	40.9	42.4	100.0	0.0	100.0	46.4	44.3	130.7
	Pentacosene	36.4	37.9	40.0	38.7	38.2	37.5	48.4	32.1		34.9	32.5	38.3
	Pentacosane												
	5 Me C23	42.1	42.1	46.5	44.6	39.5	34.9	51.8	41.1	47.1	41.7	43.0	
	Heptacosene	39.5	0.0	0.0	39.3	41.9	0.0						0.0
	5 Me C27	48.5	120.6	100.0	56.6	141.1	74.8					0.0	89.1
Percentage of M+2	Tricosane	66.9	0.0	0.0	102.4	96.1	75.5					0.0	
	5 Me C23	99.8	116.9	111.6	117.4	107.1	83.0	100.0	82.1	100.0	97.0	101.2	195.4
	Pentacosene	54.2	56.5	55.2	52.1	49.1	40.1	67.4	49.3	77.6	53.2	39.9	52.8
	Pentacosane												
	5 Me C23	85.2	90.6	104.9	96.0	91.9	70.4	133.7	95.1	142.9	85.6	88.5	
	Heptacosene	45.7	0.0	0.0	53.5	35.4	0.0						
	5 Me C27	123.6	140.6	100.0	127.8	167.1	116.0					96.6	150.6
Percentage of M+3	Tricosane	0.0	0.0	0.0	0.0	0.0	0.0					0.0	
	5 Me C23	127.8	148.5	174.4	149.7	159.0	118.1	121.5	97.7	165.9	157.4	138.8	215.4
	Pentacosene	27.8	30.0	36.3	30.2	23.2	20.5	38.1	31.1	48.2	30.8	22.1	31.7
	Pentacosane												
	5 Me C23	136.2	136.9	163.6	148.4	132.8	97.1	200.1	141.0	244.3	140.5	126.8	
	Heptacosene	0.0	0.0	0.0	26.7	0.0	0.0						0.0
	5 Me C27	193.1	217.0	118.5	203.3	185.7	122.9					169.5	173.8
Percentage of M+4	Tricosane	69.5	110.3	0.0	121.5	76.3	0.0					51.5	
	5 Me C23	165.9	179.4	197.6	173.3	140.9	106.0	131.5	141.3	192.0	174.2	145.5	206.7
	Pentacosene	47.4	51.0	58.7	48.4	37.2	32.8	62.3	43.0	66.8	46.6	31.4	46.5
	Pentacosane												
	5 Me C23	157.8	152.6	195.3	175.2	128.5	96.2	219.3	148.0	293.9	163.1	143.7	
	Heptacosene	44.9	0.0	0.0	46.5	37.5	0.0						51.1
	5 Me C27	223.4	233.9	226.7	240.1	215.7	101.5					154.1	243.1

0
0-20
20-40
40-60
80-100
100+

Figure C.42: Data sheet showing page three of the raw substrate data for the L-valine $^{13}C_5$,^{15}N experiment and *Myrmica sabuleti*.

L-Valine $^{13}C_5$, ^{15}N		*Myrmica sabuleti*											
		Val 1	Val 2	Val 3	Val 4	Val 5	Val 6	Val 7	Val 8	Val 9	Val 10	Val 11	Val 12
Percentage of M+5	Tricosane	0.0	0.0	0.0	0.0	0.0	0.0					0.0	
	5 Me C23	193.7	197.0	239.0	188.9	153.2	115.2	119.5	136.2	189.8	196.2	169.1	175.6
	Pentacosene	26.1	27.9	32.6	27.9	18.9	18.9	31.4	32.0	43.9	30.1	21.4	29.3
	Pentacosane												
	5 Me C23	194.2	184.2	249.2	211.5	140.2	111.2	249.4	204.5	357.5	187.3	160.5	
	Heptacosene	32.7	0.0	0.0	28.7	0.0	0.0						0.0
	5 Me C27	322.2	213.9	228.2	334.3	168.9	94.7					235.3	229.2
Percentage of M+6	Tricosane	65.8	0.0	0.0	77.5	0.0	0.0					0.0	
	5 Me C23	169.1	153.2	203.7	165.7	105.4	91.6	96.0	163.8	176.1	159.9	139.8	115.7
	Pentacosene	33.1	37.1	44.1	35.2	23.4	20.8	37.6	36.4	52.3	34.1	25.2	37.0
	Pentacosane												
	5 Me C23	179.5	150.4	216.3	181.8	106.9	81.0	185.0	181.6	299.7	155.3	131.8	
	Heptacosene	0.0	0.0	0.0	34.3	0.0	0.0						0.0
	5 Me C27	271.9	200.0	220.5	302.7	175.4	81.3					178.1	227.0
Percentage of M+7	Tricosane	0.0	0.0	0.0	0.0	0.0	0.0					0.0	
	5 Me C23	178.2	141.5	173.5	144.0	101.1	80.4	0.0	112.4	187.5	150.3	130.4	113.6
	Pentacosene	18.8	20.8	23.2	19.7	10.7	11.4	0.0	21.5	30.2	18.9	13.8	18.6
	Pentacosane												
	5 Me C23	182.7	138.2	203.9	187.9	102.8	82.2	169.8	173.6	306.7	152.5	129.5	
	Heptacosene	0.0	0.0	0.0	0.0	0.0	0.0						0.0
	5 Me C27	307.3	170.9	260.0	300.9	95.7	0.0					189.4	164.8
Percentage of M+8	Tricosane	0.0	0.0	0.0	0.0	0.0	0.0					0.0	
	5 Me C23	128.1	88.1	124.7	110.3	57.4	45.9	0.0	97.7	130.7	101.1	80.2	95.0
	Pentacosene	19.7	18.5	23.9	18.9	11.7	11.3	0.0	24.4	30.7	18.3	13.9	20.0
	Pentacosane												
	5 Me C23	135.8	94.6	146.9	126.4	61.5	49.7	101.6	132.2	357.5	103.6	87.4	
	Heptacosene	0.0	0.0	0.0	0.0	0.0	0.0						0.0
	5 Me C27	215.4	137.0	195.9	228.7	86.1	0.0					159.6	148.3
Percentage of M+9	Tricosane	0.0	0.0	0.0	0.0	0.0	0.0					0.0	
	5 Me C23	109.3	61.4	90.2	76.8	33.5	40.0	0.0	72.5	0.0	76.9	75.0	57.2
	Pentacosene	10.3	9.9	11.7	10.7	5.6	5.6	0.0	9.9	0.0	9.4	7.6	10.8
	Pentacosane												
	5 Me C23	116.9	72.3	119.5	106.0	52.3	40.1	78.6	97.5	216.6	84.6	75.4	
	Heptacosene	0.0	0.0	0.0	0.0	0.0	0.0						0.0
	5 Me C27	222.2	0.0	140.5	212.9	0.0	0.0					133.2	98.5
Percentage of M+10	Tricosane	0.0	0.0	0.0	0.0	0.0	0.0					0.0	
	5 Me C23	56.5	0.0	58.8	45.8	0.0	0.0	0.0	0.0	0.0	48.9	34.7	0.0
	Pentacosene	10.8	8.0	10.3	9.0	4.4	4.3	0.0	12.6	0.0	7.6	6.3	10.5
	Pentacosane												
	5 Me C23	74.0	45.4	68.3	62.8	28.0	23.7	39.5	65.6	175.5	49.0	44.5	
	Heptacosene	0.0	0.0	0.0	0.0	0.0	0.0						0.0
	5 Me C27	151.3	0.0	109.7	127.6	0.0	0.0					77.1	70.8
Percentage of M+11	Tricosane	0.0	0.0	0.0	0.0	0.0	0.0					0.0	
	5 Me C23	38.3	0.0	0.0	28.4	0.0	0.0	0.0	0.0	0.0	0.0	26.0	0.0
	Pentacosene	5.4	4.2	0.0	4.6	0.0	0.0	0.0	0.0	0.0	0.0	3.7	5.2
	Pentacosane												
	5 Me C23	52.2	29.2	43.2	43.3	18.7	16.9	25.5	56.0	103.4	33.5	28.1	
	Heptacosene	0.0	0.0	0.0	0.0	0.0	0.0						0.0
	5 Me C27	112.1	0.0	0.0	94.0	0.0	0.0					55.8	0.0

0
0-20
20-40
40-60
80-100
100+

Figure C.43: Data sheet showing page four of the raw substrate data for the L-valine $^{13}C_5$, ^{15}N experiment and *Myrmica sabuleti*.

L-Valine $^{13}C_5$, ^{15}N		*Myrmica sabuleti*											
		Val 1	Val 2	Val 3	Val 4	Val 5	Val 6	Val 7	Val 8	Val 9	Val 10	Val 11	Val 12
Percentage of M+12	Tricosane	0.0	0.0	0.0	0.0	0.0	0.0					0.0	
	5 Me C23	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Pentacosene	4.1	0.0	0.0	2.8	0.0	0.0	0.0	0.0	0.0	0.0	3.1	3.7
	Pentacosane												
	5 Me C23	30.5	12.9	23.5	24.3	8.2	8.1	0.0	25.6	72.0	16.5	14.4	
	Heptacosene	0.0	0.0	0.0	0.0	0.0	0.0						0.0
	5 Me C27	65.5	0.0	0.0	47.0	0.0	0.0					0.0	0.0
Percentage of M+13	Tricosane	0.0	0.0	0.0	0.0	0.0	0.0					0.0	
	5 Me C23	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Pentacosene	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Pentacosane												
	5 Me C23	22.7	13.5	18.2	18.1	10.4	9.0	0.0	20.2	38.0	13.7	13.3	
	Heptacosene	0.0	0.0	0.0	0.0	0.0	0.0						0.0
	5 Me C27	48.5	0.0	0.0	39.2	0.0	0.0					0.0	0.0
Percentage of M+14	Tricosane	0.0	0.0	0.0	0.0	0.0	0.0					0.0	
	5 Me C23	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Pentacosene	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Pentacosane												
	5 Me C23	15.1	11.4	14.7	15.3	10.9	9.3	0.0	16.0	24.9	11.8	12.0	
	Heptacosene	0.0	0.0	0.0	0.0	0.0	0.0						0.0
	5 Me C27	39.5	0.0	0.0	0.0	0.0	0.0					0.0	0.0
Percentage of M+15	Tricosane	0.0	0.0	0.0	0.0	0.0	0.0					0.0	
	5 Me C23	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Pentacosene	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Pentacosane												
	5 Me C23	11.1	7.8	8.3	10.8	6.3	5.1	0.0	11.0	17.6	8.3	8.1	
	Heptacosene	0.0	0.0	0.0	0.0	0.0	0.0						0.0
	5 Me C27	0.0	0.0	0.0	0.0	0.0	0.0					0.0	0.0

0
0-20
20-40
40-60
80-100
100+

Figure C.44: Data sheet showing page five of the raw substrate data for the L-valine $^{13}C_5$,^{15}N experiment and *Myrmica sabuleti*.

Sodium [1-^{13}C]acetate (Positive Control)		*Formica lemani*																
		SA 1	SA 2	SA 3	SA 4	SA 5	SA 6	SA 7	SA 8	SA 9	SA 10	SA 11	SA 12	SA 13	SA 14	SA 15	SA 16	SA 17
Abundance of M	Tricosene	1078	557	1246		1488			3367	3279	1695		1047	681	2949	1528	1214	1234
	Tricosane	22712	17536	22760	9023	24184			57416	60384	46224		27816	23912	29264	26008	22176	22848
	3 Me C23	1038	1485	5191	1469	1702			5010	3851	1945		3264	1237	5438	3220	2143	1163
	Pentacosene	4772	2287	6883	1324	7883			15932	17160	7962		5112	3355	15989	8064	5626	6095
	Pentacosane	10364	6827	11036	3303	13194			28352	32072	19600		12216	10644	18944	12790	10479	11384
	3 Me C25	229	422	2981	291	267			1096	1111	374		1291	332	2178	1598	574	171
	Heptacosene			5044	835	1781			5168	4299	1894		2736	1033	5718	3134	1735	1398
	Heptacosane	381		517		587			1411	1546	503		364	236	1341	596	344	404
Abundance of M+1	Tricosene	399	183	426		528			1094	1079	615		337	208	1020	551	443	442
	Tricosane	9281	7253	9202	3484	9496			22272	24072	18376		10730	10139	11272	10347	8792	9461
	3 Me C23	423	569	1859	600	708			2379	1589	920		1395	526	2365	1374	834	529
	Pentacosene	1772	815	2574	474	2775			5596	5988	2772		1790	1277	5512	2914	1989	2166
	Pentacosane	5244	3391	5570	1707	6470			15081	16536	9811		6596	5578	9527	6374	5385	6014
	3 Me C25		165	1034					632	381			589	163	950	673	238	
	Heptacosene			1746	362	642			1888	1710	751		1012	346	2124	1100	613	515
	Heptacosane	232		333		427			907	1092	450		272	195	958	422	282	337
Abundance of M+2	Tricosene	195		186		246			579	572	278		188		545	320	206	233
	Tricosane	3011	2332	3025	1269	2887			7720	7612	6218		3480	3280	3713	3421	2907	3172
	3 Me C23	202	231	873	290	363			1423	770	438		701	276	1240	590	322	299
	Pentacosene	866	301	1097	218	1172			2602	2816	1336		895	541	2532	1319	893	1093
	Pentacosane	2496	1671	2431	761	2927			6854	7537	4735		3006	2601	4245	2901	2593	2661
	3 Me C25			460					463	174			342		519	262		
	Heptacosene			880	158	303			992	949	423		468	225	1183	549	306	254
	Heptacosane	164		235		245			614	738	297		192		702	266	189	200
Abundance of M+3	Tricosene			168		157			425	353	209				390	208	163	160
	Tricosane	996	762	1040	402	936			2668	2820	1944		1270	1071	1301	1071	976	1046
	3 Me C23	155	212	759	280	245			1138	642	362		566	253	994	483	221	216
	Pentacosene	654	236	834	207	873			2087	2314	1050		610	446	1884	978	684	756
	Pentacosane	1088	716	1111	407	1197			3099	3533	2327		1393	1240	1983	1491	1131	1199
	3 Me C25			325					354				322		333	185		
	Heptacosene			704	161	223			832	582	340		430	180	920	473	224	215
	Heptacosane								361	425	181				399	183		

Figure C.45: Data sheet showing page one of the raw control data for sodium [1-^{13}C]acetate and *Formica lemani*.

Sodium [1-^{13}C]acetate (Positive Control)		*Formica lemani*																
		SA 1	SA 2	SA 3	SA 4	SA 5	SA 6	SA 7	SA 8	SA 9	SA 10	SA 11	SA 12	SA 13	SA 14	SA 15	SA 16	SA 17
Percentage of M+1	Tricosene	37.0	32.9	34.2		35.5			32.5	32.9	36.3		32.2	30.5	34.6	36.1	36.5	35.8
	Tricosane	40.9	41.4	40.4	38.6	39.3			38.8	39.9	39.8		38.6	42.4	38.5	39.8	39.6	41.4
	3 Me C23	40.8	38.3	35.8	40.8	41.6			47.5	41.3	47.3		42.7	42.5	43.5	42.7	38.9	45.5
	Pentacosene	37.1	35.6	37.4	35.8	35.2			35.1	34.9	34.8		35.0	38.1	34.5	36.1	35.4	35.5
	Pentacosane	50.6	49.7	50.5	51.7	49.0			53.2	51.6	50.1		54.0	52.4	50.3	49.8	51.4	52.8
	3 Me C25	0.0	39.1	34.7	0.0	0.0			57.7	34.3	0.0		45.6	49.1	43.6	42.1	41.5	0.0
	Heptacosene			34.6	43.4	36.0			36.5	39.8	39.7		37.0	33.5	37.1	35.1	35.3	36.8
	Heptacosane	60.9		64.4		72.7			64.3	70.6	89.5		74.7	82.6	71.4	70.8	82.0	83.4
Percentage of M+2	Tricosene	18.1	0.0	14.9		16.5			17.2	17.4	16.4		18.0	0.0	18.5	20.9	17.0	18.9
	Tricosane	13.3	13.3	13.3	14.1	11.9			13.4	12.6	13.5		12.5	13.7	12.7	13.2	13.1	13.9
	3 Me C23	19.5	15.6	16.8	19.7	21.3			28.4	20.0	22.5		21.5	22.3	22.8	18.3	15.0	25.7
	Pentacosene	18.1	13.2	15.9	16.5	14.9			16.3	16.4	16.8		17.5	16.1	15.8	16.4	15.9	17.9
	Pentacosane	24.1	24.5	22.0	23.0	22.2			24.2	23.5	24.2		24.6	24.4	22.4	22.7	24.7	23.4
	3 Me C25	0.0	0.0	15.4	0.0	0.0			42.2	15.7	0.0		26.5	0.0	23.8	16.4	0.0	0.0
	Heptacosene			17.4	18.9	17.0			19.2	22.1	22.3		17.1	21.8	20.7	17.5	17.6	18.2
	Heptacosane	43.0		45.5		41.7			43.5	47.7	59.0		52.7	0.0	52.3	44.6	54.9	49.5
Percentage of M+3	Tricosene	0.0	0.0	13.5		10.6			12.6	10.8	12.3		0.0	0.0	13.2	13.6	13.4	13.0
	Tricosane	4.4	4.3	4.6	4.5	3.9			4.6	4.7	4.2		4.6	4.5	4.4	4.1	4.4	4.6
	3 Me C23	14.9	14.3	14.6	19.1	14.4			22.7	16.7	18.6		17.3	20.5	18.3	15.0	10.3	18.6
	Pentacosene	13.7	10.3	12.1	15.6	11.1			13.1	13.5	13.2		11.9	13.3	11.8	12.1	12.2	12.4
	Pentacosane	10.5	10.5	10.1	12.3	9.1			10.9	11.0	11.9		11.4	11.6	10.5	11.7	10.8	10.5
	3 Me C25	0.0	0.0	10.9	0.0	0.0			32.3	0.0	0.0		24.9	0.0	15.3	11.6	0.0	0.0
	Heptacosene			14.0	19.3	12.5			16.1	13.5	18.0		15.7	17.4	16.1	15.1	12.9	15.4
	Heptacosane	0.0		0.0		0.0			25.6	27.5	36.0		0.0	0.0	29.8	30.7	0.0	0.0

0
0-20
20-40
40-60
60-80
80-100
100+

Figure C.46: Data sheet showing page two of the raw control data for sodium [1-^{13}C]acetate and *Formica lemani*.

Sodium [1-^{13}C]acetate (Positive Control)		*Myrmica sabuleti*								
		SA 1	SA 2	SA 3	SA 4	SA 5	SA 6	SA 7	SA 8	SA 9
Abundance of M	Tricosane	951		3151	2002		717		527	
	5 Me C23	1644	243	4885	3323	496	1147	472	1116	
	Pentacosene	7723	1185	18608	13024	2601	5138	2594	8493	
	Pentacosane	15920	2256	36640	23608	2868	8330	4124	10944	
	5 Me C25	1532		3285	2319	286	573	300	1053	
	Heptacosene	1997	206	3454	2472	296	691	343	790	
	5 Me C27	731		2494	1430		523		338	
Abundance of M+1	Tricosane	886	160	3134	1742	259	697	263	716	
	5 Me C23	4475	720	12580	7379	1446	3251	1717	5613	
	Pentacosene	294		1103	463		250		165	
	Pentacosane	1052		2503	1526	218	446	216	696	
	5 Me C25	1304	177	2648	1556	250	515	273	736	
	Heptacosene	539		1874	988		394		357	
	5 Me C27	587		1998	985	151	516	208	495	
Abundance of M+2	Tricosane	2861	498	8453	4389	895	2331	1065	3997	
	5 Me C23	245		770	252		184		158	
	Pentacosene	6874	1023	18184	8719	1555	4637	2078	6564	
	Pentacosane	653		1565	820		321	181	547	
	5 Me C25	505		1616	739		368		330	
	Heptacosene	396		1537	649		322		375	
	5 Me C27	2453	372	7594	3323		2091		3405	
Abundance of M+3	Tricosane	208		668	290		174		153	
	5 Me C23	5155	764	12761	5798		3351	1656	5126	
	Pentacosene	529		1541	759		271		536	
	Pentacosane									
	5 Me C25									
	Heptacosene									
	5 Me C27									

Figure C.47: Data sheet showing page one of the raw control data for sodium [1-^{13}C]acetate and *Myrmica sabuleti*.

Sodium [1-^{13}C]acetate (Positive Control)		*Myrmica sabuleti*								
		SA 1	SA 2	SA 3	SA 4	SA 5	SA 6	SA 7	SA 8	SA 9
Percentage of M+1	Tricosane	76.9		79.1	71.4		72.9		64.1	
	5 Me C23	53.9	65.8	64.2	52.4	52.2	60.8	55.7	64.2	
	Pentacosene	57.9	60.8	67.6	56.7	55.6	63.3	66.2	66.1	
	Pentacosane	70.2		72.8	72.9		67.6		74.7	
	5 Me C25	61.1	67.6	65.7	57.1	86.5	71.9	72.1	72.3	
	Heptacosene	68.7		76.2	65.8	76.2	77.8	72.0	66.1	
	5 Me C27	65.3	85.9	76.7	62.9	84.5	74.5	79.6	93.2	
Percentage of M+2	Tricosane	56.7		59.5	49.4		55.0		67.7	
	5 Me C23	35.7	0.0	40.9	29.6	30.4	45.0	44.1	44.4	
	Pentacosene	37.0	42.0	45.4	33.7	34.4	45.4	41.1	47.1	
	Pentacosane	58.5		50.8	39.7		49.7		71.5	
	5 Me C25	43.2	45.3	49.6	36.9	54.2	55.7	50.4	60.0	
	Heptacosene	42.6		47.6	35.4	0.0	56.0	60.3	51.9	
	5 Me C27	46.6	78.2	60.2	45.2	0.0	59.3	55.1	85.3	
Percentage of M+3	Tricosane	53.1		51.3	36.9		51.3		62.6	
	5 Me C23	24.1	0.0	31.5	19.5	0.0	28.1	0.0	33.6	
	Pentacosene	31.8	31.4	40.8	25.5	0.0	40.7	0.0	40.1	
	Pentacosane	49.6		44.1	45.7		47.0		69.2	
	5 Me C25	32.4	33.9	34.8	24.6	0.0	40.2	40.2	46.8	
	Heptacosene	34.5		46.9	32.7	0.0	47.3	0.0	50.9	
	5 Me C27	40.3	0.0	46.9	33.9	0.0	52.5	50.1	58.6	

0
0-20
20-40
40-60
60-80
80-100
100+

Figure C.48: Data sheet showing page two of the raw control data for sodium [1-^{13}C]acetate and *Myrmica sabuleti*.

Appendix D

Excel data sheets of the raw data for Chapter 6

The following pages show the raw data sheets from the fatty acid substrate experiments. The cells are colour coded according to the percentage abundance calculated from the raw mass spectra data, see Section 3.4.2.1. Note that the control data was the same for all experiments and consisted of multiple *Myrmica sabuleti* samples measured during the course of this project. Please note that the data extends across several pages.

Fatty Acid Experiment

Figure D.1: Raw control data for all the fatty acid substrate experiments

Figure D.2-D.3: Raw substrate data for the tetradec-5-enoic acid-5,6-d_2 fatty acid substrate experiment

Figure D.4-D.5: Raw substrate data for the tetradec-7-enoic acid-7,8-d_2 fatty acid substrate experiment

Figure D.6: Raw substrate data for the tetradec-9-enoic acid-9,10-d_2 fatty acid substrate experiment

Figure D.7-D.8: Raw substrate data for the hexadec-5-enoic acid-5,6-d_2 fatty acid substrate experiment

Figure D.9-D.10: Raw substrate data for the hexadec-6-enoic acid-6,7-d_2 fatty acid substrate experiment

Figure D.11-D.12: Raw substrate data for the hexadec-7-enoic acid-7,8-d_2 fatty acid substrate experiment

Figure D.13-D.15: Raw substrate data for the hexadec-9-enoic acid-9,10-d_2 fatty

acid substrate experiment

Figure D.16-D.17: Raw substrate data for the hexadec-11-enoic acid-11,11-d_2 fatty acid substrate experiment

Figure D.18-D.20: Raw substrate data for the octadec-7-enoic acid-7,8-d_2 fatty acid substrate experiment

Figure D.21-D.23: Raw substrate data for the octadec-11-enoic acid-11,12-d_2 fatty acid substrate experiment

Figure D.24-D.25: Raw substrate data for the eicos-9-enoic acid-9,10-d_2 fatty acid substrate experiment

Figure D.26-D.27: Raw substrate data for the eicos-11-enoic acid-11,12-d_2 fatty acid substrate experiment

		12-1	12-2	12-3	5-1	5-2	5-3	6_1	6_2	6_6	8_1	8_5	8_7	8_8	9-1	9-2	9-3	241	243	11-1	11-2
Abundance of M	Tricosane	9511	5643	2832	6185	10702	7110	203840	25416	248256	121784	87752	185920	164992	2025	1710	4673	23136	14026	2916	3584
	Pentacosene	36272	29936	18064	55744	68224	77840	654400	99896	1143808	755520	706112	944384	1343488	21192	17016	46280	209920	168640	9535	23560
	Pentacosene : 2	710	958	1075	587	1012	953	57232	5755	187776	21016	23488	71976	69280	1013	1276	1113	10994	8298	954	4220
	Pentacosane	5528	3051	1449	2047	3250	2291	70536	4379	113264	41656	23736	90480	107592	817	621	2291	10726	6233	1358	1711
	11 13 Me C25	10459	9986	6713	9873	16320	18168	133696	13282	237376	115776	102440	148288	187840	7443	5204	14540	48344	51456	2385	5019
	5 Me C25	43784	35128	15805	54016	69576	66512	672448	89432	1191424	745536	619392	906688	1157632	17088	14218	38352	198720	142080	13233	32160
	Heptacosene	6872	4916	3206	8311	11272	12588	80976	9438	170816	130664	96448	194560	233152	4194	3173	10564	39624	33704	1246	4043
Abundance of M+1	Tricosane	2593	1263	684	1196	2595	1965	44648	7185	74456	31312	16928	35376	48624	465	345	1541	5879	4378	689	994
	Pentacosene	9282	7836	5408	14627	17808	19824	176768	27176	302016	202944	160320	237632	369024	5257	4244	11910	52584	45080	2644	7232
	Pentacosene : 2	171	183	444	192	272	271	16784	1192	48720	3841	11626	21368	19984	171	252	308	2681	2149	197	1262
	Pentacosane	1391	960	245	409	855	739	28976	1164	38344	13305	9397	25984	31816	245	198	427	1773	1596	366	602
	11 13 Me C25	5839	5178	3494	5276	8433	10204	73424	7299	127504	82712	50944	70960	99552	4100	3059	8023	25472	28440	1067	2517
	5 Me C25	9128	7295	3863	10935	16808	13464	148416	22320	261504	160704	127656	187584	252864	4336	2945	7922	44728	31752	2189	6463
	Heptacosene	2253	1597	1186	2608	3133	4395	17608	2284	49744	46848	34736	49336	65088	1230	1100	2766	12232	10353	275	1286
Abundance of M+2	Tricosane	246	155		207	225	352	5437	1312	11714	1947	4354	4432	5040			191	560	991		
	Pentacosene	1568	1255	813	2157	2008	2534	18960	2208	37920	19104	22424	33656	45696	782	598	1699	6961	6168	325	
	Pentacosene : 2							2440	479	3971	2027	2960	2797	3601				361	623		183
	Pentacosane	226						2296		4996	2753	2053	2474	4488				431	421		
	11 13 Me C25	554	791	578	652	936	1041	8778	1236	16114	7339	9018	17360	14105	468	353	958	3841	3378	192	295
	5 Me C25	1108	949	236	1531	1699	1348	19176	1840	26208	17536	14430	24192	30640	531	406	1002	4633	3121	282	810
	Heptacosene	322			447	569	700	3288	425	5303	7292	8276	3445	7955	186		563	1954	1304		171
Percentage of M+1	Tricosane	27.3	22.4	24.2	19.3	24.2	27.6	21.9	28.3	30.0	25.7	19.3	19.0	29.5	23.0	20.2	33.0	25.4	31.2	23.6	27.7
	Pentacosene	25.6	26.2	29.9	26.2	26.1	25.5	27.0	27.2	26.4	26.9	22.7	25.2	27.5	24.8	24.9	25.7	25.0	26.7	27.7	30.7
	Pentacosene : 2	24.1	19.1	41.3	32.7	26.9	28.4	29.3	20.7	25.9	18.3	49.5	29.7	28.8	16.9	19.7	27.7	24.4	25.9	20.6	29.9
	Pentacosane	25.2	31.5	16.9	20.0	26.3	32.3	41.1	26.6	33.9	31.9	39.6	28.7	29.6	30.0	31.9	18.6	16.5	25.6	27.0	35.2
	11 & 13 Me C25	55.8	51.9	52.0	53.4	51.7	56.2	54.9	55.0	53.7	71.4	49.7	47.9	53.0	55.1	58.8	55.2	52.7	55.3	44.7	50.1
	5 Me C25	20.8	20.8	24.4	20.2	24.2	20.2	22.1	25.0	21.9	21.6	20.6	20.7	21.8	25.4	20.7	20.7	22.5	22.3	16.5	20.1
	Heptacosene	32.8	32.5	37.0	31.4	27.8	34.9	21.7	24.2	29.1	35.9	36.0	25.4	27.9	29.3	34.7	26.2	30.9	30.7	22.1	31.8
Percentage of M+2	Tricosane	2.6	2.7	0.0	3.3	2.1	5.0	2.7	5.2	4.7	1.6	5.0	2.4	3.1	0.0	0.0	4.1	2.4	7.1	0.0	0.0
	Pentacosene	4.3	4.2	4.5	3.9	2.9	3.3	2.9	2.2	3.3	2.5	3.2	3.6	3.4	3.7	3.5	3.7	3.3	3.7	3.4	0.0
	Pentacosene : 2	0.0	0.0	0.0	0.0	0.0	0.0	4.3	8.3	2.1	9.6	12.6	3.9	5.2	0.0	0.0	0.0	3.3	7.5	0.0	4.3
	Pentacosane	4.1	0.0	0.0	0.0	0.0	0.0	3.3	0.0	4.4	6.6	8.6	2.7	4.2	0.0	0.0	0.0	4.0	6.8	0.0	0.0
	11 13 Me C25	5.3	7.9	8.6	6.6	5.7	5.7	6.6	9.3	6.8	6.3	8.8	11.7	7.5	6.3	6.8	6.6	7.9	6.6	8.1	5.9
	5 Me C25	2.5	2.7	1.5	2.8	2.4	2.0	2.9	2.1	2.2	2.4	2.3	2.7	2.6	3.1	2.9	2.6	2.3	2.2	2.1	2.5
	Heptacosene	4.7	0.0	0.0	5.4	5.0	5.6	4.1	4.5	3.1	5.6	8.6	1.8	3.4	4.4	0.0	5.3	4.9	3.9	0.0	4.2

0
0-20
20-40
40-60
60-80
80-100
100+

Figure D.1: Data sheet showing the raw control data for all the fatty acid experiments.

		Sub 1	Sub 2	Sub 3	Sub 4	Sub 5	Sub 6	Sub 7	Sub 8	Sub 9	Sub 10	Sub 11	Sub 12	Sub 13	Sub 14	Sub 15	Sub 16	Sub 17	Sub 18	Sub 19	Sub 20
Abundance of M	Tricosane	3575	6217	8708	14304	6339	3645			4003	3581	4009	5917	12393	4311	2842	8918	4044	18800	9020	5999
	Pentacosene	25896	12116	59656	121328	26144	24984			49032	51328	25760	70448	135552	47008	24680	116888	28952	214528	171264	82456
	Pentacosene : 2	1993	896	1863	5002	532	507			2801	1448	515	917	1763	1986	356	1095	836	2772	1834	1196
	Pentacosane	882	2966	3327	5494	4846	1733			1677	2488	2472	3069	4919	2388	1004	4706	2896	5282	5088	2405
	11 13 Me C25	2040	5557	10073	29328	2266	2084			6837	8026	2884	10918	24288	6182	5125	22568	5657	42400	28520	13169
	5 Me C25	15904	36136	54616	114464	21368	15878			40624	44168	19568	68032	116648	39088	24384	95496	28280	160576	155520	77088
	Heptacosene	607	1890	4903	13888	945	812			6769	7695	3158	9833	17296	5995	3117	14367	3678	24576	20864	11956
Abundance of M+1	Tricosane	1102	1905	2014	3413	2056	769			944	1089	1085	2033	3031	608	413	2007	1074	4845	3579	1946
	Pentacosene	6766	1376	15072	29376	6346	6308			13275	14340	6578	18736	41056	12941	8011	29128	6577	58088	42008	20248
	Pentacosene : 2	190	275	327	1383		241			893	808	168	184	516	793	244	182	225	962	447	267
	Pentacosane	518	868	929	1603	1802	738			1201	473	517	755	1313	840	264	1353	584	1695	1334	1108
	11 13 Me C25	2010	3221	6278	14997	1380	1390			2829	4645	2034	6512	13979	2367	2354	11158	3388	23896	15287	8076
	5 Me C25	3035	7582	11600	27680	4878	3021				9654	3990	14045	23912	7952	4713	19440	6681	34208	32264	17928
	Heptacosene	485	668	1590	4534	379	247			1981	1322	946	3171	5581	1732	712	3389	1607	6054	6109	3584
Abundance of M+2	Tricosane	165		327	538					229	254	252	412	683	423		376	240	688	873	312
	Pentacosene	1137	317	2125	3556	1024	801			2126	2413	884	2245	3683	1589	658	3906	1194	6666	5994	2342
	Pentacosene : 2			315	165		180								155					414	
	Pentacosane		266		264	741	242				299				186	197	220	153	341		276
	11 13 Me C25	191	352	607		156	221			491	645	228	176	1786	443	385	1309	161	3048		1647
	5 Me C25	358		1616	3469	712	434				993	420	1986	2506	1413	1233	2448	783	3986	4980	1412
	Heptacosene			170	925					460	554	174	214	474	443	327	820	400	1458	929	
Percentage of M+1	Tricosane	30.8	30.6	23.1	23.9	32.4	21.1			23.6	30.4	27.1	34.4	24.5	14.1	14.5	22.5	26.6	25.8	39.7	32.4
	Pentacosene	26.1	11.4	25.3	24.2	24.3	25.2			27.1	27.9	25.5	26.6	30.3	27.5	32.5	24.9	22.7	27.1	24.5	24.6
	Pentacosene : 2	9.5	30.7	17.6	27.6		47.5			31.9	55.8	32.6	20.1	29.3	39.9	68.5	16.6	26.9	34.7	24.4	22.3
	Pentacosane	58.7	29.3	27.9	29.2	37.2	42.6			71.6	19.0	20.9	24.6	26.7	35.2	26.3	28.8	20.2	32.1	26.2	46.1
	11 & 13 Me C25	98.5	58.0	62.3	51.1	60.9	66.7			41.4	57.9	70.5	59.6	57.6	38.3	45.9	49.4	59.9	56.4	53.6	61.3
	5 Me C25	19.1	21.0	21.2	24.2	22.8	19.0			0.0	21.9	20.4	20.6	20.5	20.3	19.3	20.4	23.6	21.3	20.7	23.3
	Heptacosene	79.9	35.3	32.4	32.6	40.1	30.4			29.3	17.2	30.0	32.2	32.3	28.9	22.8	23.6	43.7	24.6	29.3	30.0
Percentage of M+2	Tricosane	4.6	0.0	3.8	3.8	0.0	0.0			5.7	7.1	6.3	7.0	5.5	9.8	0.0	4.2	5.9	3.7	9.7	5.2
	Pentacosene	4.4	2.6	3.6	2.9	3.9	3.2			4.3	4.7	3.4	3.2	2.7	3.4	2.7	3.3	4.1	3.1	3.5	2.8
	Pentacosene : 2	0.0	0.0	16.9	3.3	0.0	35.5			0.0	0.0	0.0	0.0	0.0	7.8	0.0	0.0	0.0	0.0	22.6	0.0
	Pentacosane	0.0	9.0	0.0	4.8	15.3	14.0			0.0	12.0	0.0	0.0	0.0	7.8	19.6	4.7	5.3	6.5	0.0	11.5
	11 13 Me C25	9.4	6.3	6.0	0.0	6.9	10.6			7.2	8.0	7.9	1.6	7.4	7.2	7.5	5.8	2.8	7.2	0.0	12.5
	5 Me C25	2.3	0.0	3.0	3.0	3.3	2.7			0.0	2.2	2.1	2.9	2.1	3.6	5.1	2.6	2.8	2.5	3.2	1.8
	Heptacosene	0.0	0.0	3.5	6.7	0.0	0.0			6.8	7.2	5.5	2.2	2.7	7.4	10.5	5.7	10.9	5.9	4.5	0.0

0
0-20
20-40
40-60
60-80
80-100
100+

Figure D.2: Data sheet showing page one of the raw substrate data for the tetradec-5-enoic acid-5,6-d_2 fatty acid experiment.

		Sub 21	Sub 22	Sub 23	Sub 24	Sub 25	Sub 26	Sub 27	Sub 28	Sub 29	Sub 30	Sub 31	Sub 32	Sub 33	Sub 34	Sub 35	Sub 36	Sub 37
Abundance of M	Tricosane	12036	24648	12905	12915	30768	16344		38816	10289	16792	10703	5389		12181	10149	22192	23456
	Pentacosene	172928	460096	265728	126128	462016	216768		713216	61912	138624	59323	47024		214592	45584	177920	259200
	Pentacosene : 2	2382	3312	1827	1434	3464	1616		7410	1322	887	1519	4040		2963	3380	1836	1674
	Pentacosane	7520	12578	6972	6963	11833	6522		20368	7963	12705	4373	3906		5505	6264	7009	6663
	11 13 Me C25	29344	85392	42672	17600	78136	34096		110016	8912	19240	10086	7670		36360	6126	37464	52648
	5 Me C25	121920	349376	219584	98264	330368	166976		543616	60608	136768	52856	35824		181248	41736	155264	202176
	Heptacosene	16138	60472	34712	15874	55592	22896		88864	10641	17720	5865	3982		28752	6388	28880	31112
Abundance of M+1	Tricosane	2984	4937	2691	4301	7746	5108		10334	3275	4596	2613	1542		3767	3017	6026	5958
	Pentacosene	44656	110152	64944	32368	119336	54552		194176	16051	35536	14116	10992		58544	12222	53232	66016
	Pentacosene : 2	961	362	638	369	822	434		3406	238	152	477	325		763	1316	933	387
	Pentacosane	2436	3142	1813	2134	3057	1665		4155	2230	2233	1049	975		1418	1186	2416	2691
	11 13 Me C25	17368	44120	23200	10637	39072	17944		56816	4918	12679	3364	4442		19672	3618	19536	25496
	5 Me C25	25896	77552	45664	21768	74568	37304		117336	14715	26408	9800	7607		40000	9393	32784	42832
	Heptacosene	4332	15313	10168	4999	18576	7608		22728	2327	6986	2023	1589		7822	1078	6522	9088
Abundance of M+2	Tricosane		418	446	220	1490	519		1036	339	222		247		395	517	869	1450
	Pentacosene	5199	15855	7619	4292	16976	5384		24096	1979	7264	201	1312		7866	1518	5994	9977
	Pentacosene : 2		501	566		1110			851		634	340						
	Pentacosane		367	264	493	325	307		357	753		293			173	170	264	826
	11 13 Me C25	580	5006	3188	912	7573	1794		7157		1534	795	646		2466	441	2882	4194
	5 Me C25	3349	7887	5685	2969	7102	3628		12695	1521	3332	949	1387		4539	1142	3613	5005
	Heptacosene	737	1335	1140	1337	3634	2123		4076	324	702	403	246		925		1078	
Percentage of M+1	Tricosane	24.8	20.0	20.9	33.3	25.2	31.3		26.6	31.8	27.4	24.4	28.6		30.9	29.7	27.2	25.4
	Pentacosene	25.8	23.9	24.4	25.7	25.8	25.2		27.2	25.9	25.6	23.8	23.4		27.3	26.8	29.9	25.5
	Pentacosene : 2	40.3	10.9	34.9	25.7	23.7	26.9		46.0	18.0	17.1	31.4	8.0		25.8	38.9	50.8	23.1
	Pentacosane	32.4	25.0	26.0	30.6	25.8	25.5		20.4	28.0	17.6	24.0	25.0		25.8	18.9	34.5	40.4
	11 & 13 Me C25	59.2	51.7	54.4	60.4	50.0	52.6		51.6	55.2	65.9	33.4	57.9		54.1	59.1	52.1	48.4
	5 Me C25	21.2	22.2	20.8	22.2	22.6	22.3		21.6	24.3	19.3	18.5	21.2		22.1	22.5	21.1	21.2
	Heptacosene	26.8	25.3	29.3	31.5	33.4	33.2		25.6	21.9	39.4	34.5	39.9		27.2	16.9	22.6	29.2
Percentage of M+2	Tricosane	0.0	1.7	3.5	1.7	4.8	3.2		2.7	3.3	1.3	0.0	4.6		3.2	5.1	3.9	6.2
	Pentacosene	3.0	3.4	2.9	3.4	3.7	2.5		3.4	3.2	5.2	0.3	2.8		3.7	3.3	3.4	3.8
	Pentacosene : 2	0.0	15.1	31.0	0.0	32.0	0.0		11.5	0.0	71.5	22.4	0.0		0.0	0.0	0.0	0.0
	Pentacosane	0.0	2.9	3.8	7.1	2.7	4.7		1.8	9.5	0.0	6.7	0.0		3.1	2.7	3.8	12.4
	11 13 Me C25	2.0	5.9	7.5	5.2	9.7	5.3		6.5	0.0	8.0	7.9	8.4		6.8	7.2	7.7	8.0
	5 Me C25	2.7	2.3	2.6	3.0	2.1	2.2		2.3	2.5	2.4	1.8	3.9		2.5	2.7	2.3	2.5
	Heptacosene	4.6	2.2	3.3	8.4	6.5	9.3		4.6	3.0	4.0	6.9	6.2		3.2	0.0	3.7	0.0

0
0-20
20-40
40-60
60-80
80-100
100+

Figure D.3: Data sheet showing page two of the raw substrate data for the tetradec-5-enoic acid-5,6-d_2 fatty acid experiment.

		Sub 1	Sub 2	Sub 3	Sub 4	Sub 5	Sub 6	Sub 7	Sub 8	Sub 9	Sub 10	Sub 11	Sub 12	Sub 13	Sub 14	Sub 15	Sub 16	Sub 17	Sub 18	Sub 19	Sub 20	Sub 21	Sub 22	Sub 23	Sub 24	Sub 25	Sub 26
Abundance of M	Tricosane	18000	10920	6015	11443	19008	6367	20864	20208	4615	3789	45344	8151	5004	26912	18056	23832	16019	11614	15066	15548	6957	9441	26880	35776	19720	12886
	Pentacosene	129072	156096	53888	95080	319040	48000	246720	194944	36560	33256	533440	84264	68792	304384	186048	294656	176320	123464	136320	164800	94224	214592	352896	321664	243648	157696
	Pentacosene : 2	1127	1173	1023	1212	2254	921	1571	1034	534	1394	28368	6817	1889	37216	13347	10708	9797	5949	6615	7246	3340	4565	3167	2661	7579	3378
	Pentacosane	9105	4773	2877	6982	7954	3424	11024	8752	3913	2172	18304	4296	3970	11961	8617	9368	8322	6437	8582	7868	5214	28456	13826	17952	10910	5747
	11 13 Me C25	23656	32584	5995	11945	62184	5905	224448	30216	2784	2932	76816	9355	13065	41976	31776	47600	31616	13780	20824	23936	11976	30224	48544	52064	29912	25504
	5 Me C25	130368	149888	43896	93880	274688	35400	192640	159488	32320	29768	427136	63720	55288	231168	166976	226560	134400	97936	115312	133312	79920	183744	288448	235392	183424	127864
	Heptacosene	18080	24192	3841	1890	46696	6364	36928	24856	23338	3514	70312	8758	6452	38984	25184	34672	21368	12807	20936	22592	13058	28592	42480	43912	36536	21760
Abundance of M+1	Tricosane	4316	2444	1602	2308	5341	835	6097	4877	2029	717	11283	2119	1519	5058	3575	6524	3767	2442	4239	4661	1403	2813	7540	8554	5420	2777
	Pentacosene	33680	41088	13671	30128	84552	12777	61320	52120	11481	7868	141184	17280	16592	84680	50032	81624	50416	30456	39440	45584	27568	54328	94632	81560	64448	40424
	Pentacosene : 2	449	563	362	564	970	507	892	205		713	8533	1245	893	9585	6285	3348	3184	2034	2369	2165	723	2374	994	854	2781	1120
	Pentacosane	1634	1803	662	2145	3709	507	2788	1800	1805	1150	6277	2536	1608	3663	2002	3660	2141	2343	2544	3306	1824	17976	2396	3893	2305	1878
	11 13 Me C25	14034	17064	2449	5668	29544	3355	41176	17256	2390	1908	37344	4866	7243	30400	16201	25552	15055	8719	10098	15828	7036	18136	26688	26568	20032	12422
	5 Me C25	30952	38576	10503	16072	54520	7304	41336	35024	6521	7583	93384	14895	9666	47392	34400	48496	33456	20456	24768	29952	18296	41200	60368	53904	38008	29096
	Heptacosene	6402	7127	1557	668	16392	2420	9690	6913	1866	1355	20112	2861	1566	9921	9101	9269	6809	4913	5482	5085	3577	8089	14349	10603	9081	6531
Abundance of M+2	Tricosane	835	394	491	413	1246	578	750	340	320	277	1837	167	178	863	437	326	868	275	880	247	322	519	756	2086	904	312
	Pentacosene	4837	5946	1922	3382	11585	1818	9367	7167	1343	1700	16408	3087	2268	11013	6818	9817	4783	4733	4777	6087	3343	9769	13297	9311	7804	4829
	Pentacosene : 2		245		272	161		185				804	154		1643	327	818	457	380		191	288	164	554		282	
	Pentacosane	700	314		509	309	244	726			164	484	214	163	772	306	366	503	351		239		1953	2035	718	663	661
	11 13 Me C25	2494	2403	686	1267	5039	287	5011	2131	521	328	4305	458		3349	2449	1963	2343	655	779	1330	1195	1715	3201	2672	2332	1612
	5 Me C25	2815	4241	1532	2235	5944	537	6001	3851	989	475	10939	1759	1409	4466	4517	4095	2267	2014	2244	3607	2404	3571	6184	5630	5395	3147
	Heptacosene	905	974	259		2141	207	1966	903	353	804	3479	847	248	1314	1211	1111	1250	1195	1305	1388	903	1454	2505	2318	1669	1330
Percentage of M+1	Tricosane	24.0	22.4	26.6	20.2	28.1	13.1	29.2	24.1	44.0	18.9	24.9	26.0	30.4	18.8	19.8	27.4	23.5	21.0	28.1	30.0	20.2	29.8	28.1	23.9	27.5	21.6
	Pentacosene	26.1	26.3	25.4	31.7	26.5	26.6	24.9	26.7	31.4	23.7	26.5	20.5	24.1	27.8	26.9	27.7	28.6	24.7	28.9	27.7	29.3	25.3	26.8	25.4	26.5	25.6
	Pentacosene : 2	39.8	48.0	35.4	46.5	43.0	55.0	56.8	19.8		51.1	30.1	18.3	47.3	25.8	47.1	31.3	32.5	34.2	35.8	29.9	21.6	52.0	31.4	32.1	36.7	33.2
	Pentacosane	17.9	37.8	23.0	30.7	46.6	14.8	25.3	20.6	46.1	52.9	34.3	59.0	40.5	30.6	23.2	39.1	25.7	36.4	29.6	42.0	35.0	63.2	17.3	21.7	21.1	32.7
	11 & 13 Me C25	59.3	52.4	40.9	47.5	47.5	56.8	18.3	57.1	85.8	65.1	48.6	52.0	55.4	72.4	51.0	53.7	47.6	63.3	48.5	66.1	58.8	60.0	55.0	51.0	67.0	48.7
	5 Me C25	23.7	25.7	23.9	17.1	19.8	20.6	21.5	22.0	20.2	25.5	21.9	23.4	17.5	20.5	20.6	21.4	24.9	20.9	21.5	22.5	22.9	22.4	20.9	22.9	20.7	22.8
	Heptacosene	35.4	29.5	40.5	35.3	35.1	38.0	26.2	27.8	8.0	38.6	28.6	32.7	24.3	25.4	36.1	26.7	31.9	38.4	26.2	22.5	27.4	28.3	33.8	24.1	24.9	30.0
Percentage of M+2	Tricosane	4.6	3.6	8.2	3.6	6.6	9.1	3.6	1.7	6.9	7.3	4.1	2.0	3.6	3.2	2.4	1.4	5.4	2.4	5.8	1.6	4.6	5.5	2.8	5.8	4.6	2.4
	Pentacosene	3.7	3.8	3.6	3.6	3.6	3.8	3.8	3.7	3.7	5.1	3.1	3.7	3.3	3.6	3.7	3.3	2.7	3.8	3.5	3.7	3.5	4.6	3.8	2.9	3.2	3.1
	Pentacosene : 2	0.0	20.9	0.0	22.4	7.1	0.0	11.8	0.0	0.0	0.0	2.8	2.3	0.0	4.4	2.4	7.6	4.7	6.4	0.0	2.6	8.6	3.6	17.5	0.0	3.7	0.0
	Pentacosane	7.7	6.6	0.0	7.3	3.9	7.1	6.6	0.0	0.0	7.6	2.6	5.0	4.1	6.5	3.6	3.9	6.0	5.5	0.0	3.0	0.0	6.9	14.7	4.0	6.1	11.5
	11 13 Me C25	10.5	7.4	11.4	10.6	8.1	4.9	2.2	7.1	18.7	11.2	5.6	4.9	0.0	8.0	7.7	4.1	7.4	4.8	3.7	5.6	10.0	5.7	6.6	5.1	7.8	6.3
	5 Me C25	2.2	2.8	3.5	2.4	2.2	1.5	3.1	2.4	3.1	1.6	2.6	2.8	2.5	1.9	2.7	1.8	1.7	2.1	1.9	2.7	3.0	1.9	2.1	2.4	2.9	2.5
	Heptacosene	5.0	4.0	6.7	0.0	4.6	3.3	5.3	3.6	1.5	22.9	4.9	9.7	3.8	3.4	4.8	3.2	5.8	9.3	6.2	6.1	6.9	5.1	5.9	5.3	4.6	6.1

0
0-20
20-40
40-60
60-80
80-100
100+

Figure D.4: Data sheet showing page one of the raw substrate data for the tetradec-7-enoic acid-7,8-d_2 fatty acid experiment.

		Sub 27	Sub28	Sub29	Sub30	Sub31	Sub32	Sub33	Sub34	Sub35	Sub36	Sub37	Sub38	Sub39	Sub40	Sub41	Sub42	Sub43	Sub44	Sub45	Sub46	Sub47	Sub48	Sub49	Sub50	Sub51	Sub52	Sub53
Abundance of M	Tricosane	6651	7589	10495	14045	22360	121840	17200	20640	38888	20448	29136	50864	56728	30424	37584	47872	26616	21376	34224	20912	28888	31368	29248	38328	39992	33392	61856
	Pentacosene	85816	82416	102448	107384	246912	1368064	188864	272064	365568	163072	305280	583104	605504	380288	304576	479488	253760	147328	337856	292160	286400	302080	353408	385664	518080	383552	417728
	Pentacosene : 2	1131	2285	1515	1796	1411	33680	1428	1365	2614	3557	2986	5857	17392	23272	2831	12978	10197	2590	2253	4696	7124	7286	2928	7163	5216	2880	4643
	Pentacosane	4727	3720	5735	6870	11775	61000	8818	9179	20816	11188	10854	26808	25616	19704	23416	19680	13593	13904	16048	9077	14444	15588	14662	24296	17048	20808	36712
	11 13 Me C25	10973	10655	17480	16496	33232	160128	26184	37128	56568	18616	65240	82288	89000	59528	48304	71392	38104	13716	54904	56256	34384	38848	46720	51992	80792	47472	54136
	5 Me C25	68616	68552	89728	86896	212608	1032832	149760	225344	278080	122032	266304	441920	410176	327360	277120	395904	188096	114080	253568	258880	202240	240320	281984	315968	378560	301248	321792
	Heptacosene	12123	10677	17136	14120	36928	188736	25648	37928	47968	23352	40688	84040	78488	56176	46824	70744	34512	20048	48672	45328	37200	41184	47328	56392	65888	50856	62400
Abundance of M+1	Tricosane	1862	1826	2797	4296	6815	27328	5229	4918	11849	4383	5811	12814	12803	7686	11044	12610	6422	4995	8702	5447	8705	7107	6483	8415	9314	9134	16167
	Pentacosene	20600	21000	27496	28264	63136	369472	56352	76448	93272	47256	88328	154432	156992	107464	78032	132096	60624	35816	91176	82256	70312	81584	92960	109936	130680	96984	108000
	Pentacosene : 2	353	1101	485	758	631	11736	752	569	1215	1031	1411	2381	5813	7582	1518	3350	4255	810	979	1676	2985	1290	1245	1923	1532	870	1721
	Pentacosane	1171	1360	1769	1570	3581	19000	2136	1703	7231	2374	2994	7550	7069	3343	5020	5239	4285	4667	5184	1994	3738	3847	3607	4945	5381	8531	10781
	11 13 Me C25	4551	5294	8723	8763	15048	86136	16286	19528	26808	8263	33160	41968	49784	29296	25840	38264	19704	9030	25944	26512	16736	22400	20368	23552	39088	23912	28896
	5 Me C25	13412	13366	19368	15238	46856	221568	32904	46552	61064	30128	55272	91632	87152	70208	62480	84576	42456	24560	51712	51864	40280	47640	63280	71968	80112	67808	75448
	Heptacosene	3385	3899	4372	4111	11087	56720	8903	12766	13730	6702	12109	24016	20896	18024	12655	20400	10662	5555	13707	12048	11989	11480	17680	17224	17544	14988	15923
Abundance of M+2	Tricosane	283	413	239	503		4522	297	523	1787	875	682	1940	1494	1164	1315	1444	1447	826	1389	375	1530	527	490	1256	1896	973	3247
	Pentacosene	3118	3058	3493	4164	9243	45056	6428	11030	11554	5153	10297	19192	20504	12599	10952	16323	7603	4662	8385	9374	7631	8478	10674	12809	17240	15139	12099
	Pentacosene : 2						2825	166		573	294	647	263	1179	1516	278	988	505	585	337		317	214	211	170	685	333	515
	Pentacosane			160	299	179	2401		538	751	452	697	1263	952	925	622	826	737		642		408	429	1263	981	1409	776	1778
	11 13 Me C25	750			1569	3211	11681	2234	2871	3134	1578	5062	6630	5943	3793	4637	4492	2900	1684	4927	3057	1466	3350	2215	3106	4901	3618	4402
	5 Me C25	1727	1667	2504	1563	5809	25704	3586	5739	6336	4406	6503	10651	8590	7131	5698	10581	4217	2903	5264	4899	6889	5442	7990	7942	7910	6659	8409
	Heptacosene	530	408	746	750	1744	6674	1439	1515	2438	1443	1558	2970	468	2919	2062	2499	1359	1317	1626	2273	1646	930	3144	2661	2721	1755	1322
Percentage of M+1	Tricosane	28.0	24.1	26.7	30.6	30.5	22.4	30.4	23.8	30.5	21.4	19.9	25.2	22.6	25.3	29.4	26.3	24.1	23.4	25.4	26.0	30.1	22.7	22.2	22.0	23.3	27.4	26.1
	Pentacosene	24.0	25.5	26.8	26.3	25.6	27.0	29.8	28.1	25.5	29.0	28.9	26.5	25.9	28.3	25.6	27.5	23.9	24.3	27.0	28.2	24.6	27.0	26.3	28.5	25.2	25.3	25.9
	Pentacosene : 2	31.2	48.2	32.0	42.2	44.7	34.8	52.7	41.7	46.5	29.0	47.3	40.7	33.4	32.6	53.6	25.8	41.7	31.3	43.5	35.7	41.9	17.7	42.5	26.8	29.4	30.2	37.1
	Pentacosane	24.8	36.6	30.8	22.9	30.4	31.1	24.2	18.6	34.7	21.2	27.6	28.2	27.6	17.0	21.4	26.6	31.5	33.6	32.3	22.0	25.9	24.7	24.6	20.4	31.6	41.0	29.4
	11 & 13 Me C25	41.5	49.7	49.9	53.1	45.3	53.8	62.2	52.6	47.4	44.4	50.8	51.0	55.9	49.2	53.5	53.6	51.7	65.8	47.3	47.1	48.7	57.7	43.6	45.3	48.4	50.4	53.4
	5 Me C25	19.5	19.5	21.6	17.5	22.0	21.5	22.0	20.7	22.0	24.7	20.8	20.7	21.2	21.4	22.5	21.4	22.6	21.5	20.4	20.0	19.9	19.8	22.4	22.8	21.2	22.5	23.4
	Heptacosene	27.9	36.5	25.5	29.1	30.0	30.1	34.7	33.7	28.6	28.7	29.8	28.6	26.6	32.1	27.0	28.8	30.9	27.7	28.2	26.6	32.2	27.9	37.4	30.5	26.6	29.5	25.5
Percentage of M+2	Tricosane	4.3	5.4	2.3	3.6	0.0	3.7	1.7	2.5	4.6	4.3	2.3	3.8	2.6	3.8	3.5	3.0	5.4	3.9	4.1	1.8	5.3	1.7	1.7	3.3	4.7	2.9	5.2
	Pentacosene	3.6	3.7	3.4	3.9	3.7	3.3	3.4	4.1	3.2	3.2	3.4	3.3	3.4	3.3	3.6	3.4	3.0	3.2	2.5	3.2	2.7	2.8	3.0	3.3	3.3	3.9	2.9
	Pentacosene : 2	0.0	0.0	0.0	0.0	0.0	8.4	11.6	0.0	21.9	8.3	21.7	4.5	6.8	6.5	9.8	7.6	5.0	22.6	15.0	0.0	4.4	2.9	7.2	2.4	13.1	11.6	11.1
	Pentacosane	0.0	0.0	2.8	4.4	1.5	3.9	0.0	5.9	3.6	4.0	6.4	4.7	3.7	4.7	2.7	4.2	5.4	0.0	4.0	0.0	2.8	2.8	8.6	4.0	8.3	3.7	4.8
	11 13 Me C25	6.8	0.0	0.0	9.5	9.7	7.3	8.5	7.7	5.5	8.5	7.8	8.1	6.7	6.4	9.6	6.3	7.6	12.3	9.0	5.4	4.3	8.6	4.7	6.0	6.1	7.6	8.1
	5 Me C25	2.5	2.4	2.8	1.8	2.7	2.5	2.4	2.5	2.3	3.6	2.4	2.4	2.1	2.2	2.1	2.7	2.2	2.5	2.1	1.9	3.4	2.3	2.8	2.5	2.1	2.2	2.6
	Heptacosene	4.4	3.8	4.4	5.3	4.7	3.5	5.6	4.0	5.1	6.2	3.8	3.5	0.6	5.2	4.4	3.5	3.9	6.6	3.3	5.0	4.4	2.3	6.6	4.7	4.1	3.5	2.1

0
0-20
20-40
40-60
60-80
80-100
100+

Figure D.5: Data sheet showing page two of the raw substrate data for the tetradec-7-enoic acid-7,8-d_2 fatty acid experiment.

		Sub 1	Sub 2	Sub 3	Sub 4	Sub 5	Sub 6	Sub 7	Sub 8	Sub 9	Sub 10	Sub 11	Sub 12	Sub 13	Sub 14	Sub 15	Sub 16	Sub 17	Sub 18	Sub 19	Sub 20	Sub 21	Sub 22	Sub 23	Sub 24	Sub 25
Abundance of M	Tricosane	7588	6019	6373	87888	20328	14126	18328	37760	28680	63424	18920	27176	25184	9748	13916	12168	4938	6444	6723	51528	29672	18472	7212	4695	7234
	Pentacosene	32776	22352	28032	511104	74368	79368	124800	185408	164288	392768	195008	157568	265152	58728	92032	80136	76192	74608	43552	439104	355328	220288	71776	46976	51208
	Pentacosene : 2	1343	1451	1253	40176	2392	2152	4953	8548	6805	25200	4084	2330	6548	1456	1813	1367	1560	2367	1035	9735	4843	2729	1091	1171	1834
	Pentacosane	2732	1787	1993	31120	8462	6099	6251	17200	9851	25360	8369	8574	15514	4555	6295	4511	1742	3893	1518	23096	10542	7791	4229	2919	3670
	11 13 Me C25	2895	1977	1000	99208	5629	5614	14935	22152	25120	62888	20488	20448	36072	6234	12306	11664	11330	13795	5802	64704	58672	39496	10433	6377	9245
	5 Me C25	16384	8946	8023	463104	40672	47408	95680	146112	140608	310848	123584	109488	179072	40752	60792	59080	58144	57976	27088	331904	248832	159808	56016	43392	41856
	Heptacosene	571			81176	2535	2362	11036	17688	21008	62720	23640	23000	33936	8987	10672	10443	9301	8677	4179	58344	42512	26664	8324	5968	5556
Abundance of M+1	Tricosane	2160	1727	2073	20568	5427	3597	4431	10071	6454	14533	4317	5818	7767	2026	2734	3025	1274	2746	1235	11365	9903	4171	2457	1464	2372
	Pentacosene	8042	7843	6304	134272	21160	22016	30088	50408	47168	110512	47104	45384	70184	17840	22256	19088	20600	16880	13084	115816	91376	59264	23400	11491	13118
	Pentacosene : 2	523	407	406	9617	890	922	2339	2156	3468	6793	918	878	2132	771	285	202	446	866	304	2240	1120	746	359	314	746
	Pentacosane	898	184	425	10473	2575	1861	2229	4754	2354	5709	3192	4083	3635	2230	1629	1515	388	1066	518	7042	2377	2025	1255	790	850
	11 13 Me C25	1473	1046	629	55432	3458	3752	6835	13951	15886	36304	11417	10318	19472	3525	5651	6353	7163	8253	3202	34752	26680	19368	4319	4614	5087
	5 Me C25	4070	2837	2323	99440	10233	10466	22000	31344	29848	72104	21840	25360	36208	11325	13436	14130	14049	11249	7714	78064	54736	36224	12758	9099	8201
	Heptacosene	219			24128	403	1084	3414	5023	5809	18568	6756	7939	10593	2093	3362	2596	3034	2101	1537	17984	12925	8892	2908	1661	1753
Abundance of M+2	Tricosane	360	273	196	2507	407	537	305	1691	968	1350	289	199	1222	685	652	382		227	259	1658	780	489	292		238
	Pentacosene	1019	1348	828	15926	3526	1913	4201	7554	6613	12859	6272	4784	10247	2720	3517	3003	2085	1879	1102	15316	10697	7921	2837	1427	2247
	Pentacosene : 2				779			325	634	244	1556			188				272			314	251				160
	Pentacosane	184		190	1328	539	216	467	516	647	980	566	487	709	250		249				836	519	319	313		293
	11 13 Me C25			174	7327	479	697	951	1414	1061	4388	1312	1756	1867	616	727	929	1081	973	1090	3195	4033	3176	1055	567	785
	5 Me C25	446	237	211	11617	699	1859	2504	4885	3972	6979	3959	1670	4694	1025	1966	1729	1122	1584	1288	6952	6025	4366	1491	1201	1097
	Heptacosene				4806			903	1632	865	3083	968	1042	1316	732	1027	293	714	403		2117	1634	1347	891	174	286
Percentage of M+1	Tricosane	28.5	28.7	32.5	23.4	26.7	25.5	24.2	26.7	22.5	22.9	22.8	21.4	30.8	20.8	19.6	24.9	25.8	42.6	18.4	22.1	33.4	22.6	34.1	31.2	32.8
	Pentacosene	24.5	35.1	22.5	26.3	28.5	27.7	24.1	27.2	28.7	28.1	24.2	28.8	26.5	30.4	24.2	23.8	27.0	22.6	30.0	26.4	25.7	26.9	32.6	24.5	25.6
	Pentacosene : 2	38.9	28.0	32.4	23.9	37.2	42.8	47.2	25.2	51.0	27.0	22.5	37.7	32.6	53.0	15.7	14.8	28.6	36.6	29.4	23.0	23.1	27.3	32.9	26.8	40.7
	Pentacosane	32.9	10.3	21.3	33.7	30.4	30.5	35.7	27.6	23.9	22.5	38.1	47.6	23.4	49.0	25.9	33.6	22.3	27.4	34.1	30.5	22.5	26.0	29.7	27.1	23.2
	11 & 13 Me C25	50.9	52.9	62.9	55.9	61.4	66.8	45.8	63.0	63.2	57.7	55.7	50.5	54.0	56.5	45.9	54.5	63.2	59.8	55.2	53.7	45.5	49.0	41.4	72.4	55.0
	5 Me C25	24.8	31.7	29.0	21.5	25.2	22.1	23.0	21.5	21.2	23.2	17.7	23.2	20.2	27.8	22.1	23.9	24.2	19.4	28.5	23.5	22.0	22.7	22.8	21.0	19.6
	Heptacosene	38.4			29.7	15.9	45.9	30.9	28.4	27.7	29.6	28.6	34.5	31.2	23.3	31.5	24.9	32.6	24.2	36.8	30.8	30.4	33.3	34.9	27.8	31.6
Percentage of M+2	Tricosane	4.7	4.5	3.1	2.9	2.0	3.8	1.7	4.5	3.4	2.1	1.5	0.7	4.9	7.0	4.7	3.1	0.0	3.5	3.9	3.2	2.6	2.6	4.0	0.0	3.3
	Pentacosene	3.1	6.0	3.0	3.1	4.7	2.4	3.4	4.1	4.0	3.3	3.2	3.0	3.9	4.6	3.8	3.7	2.7	2.5	2.5	3.5	3.0	3.6	4.0	3.0	4.4
	Pentacosene : 2	0.0	0.0	0.0	1.9	0.0	0.0	6.6	7.4	3.6	6.2	0.0	0.0	2.9	0.0	0.0	0.0	17.4	0.0	0.0	3.2	5.2	0.0	0.0	0.0	8.7
	Pentacosane	6.7	0.0	9.5	4.3	6.4	3.5	7.5	3.0	6.6	3.9	6.8	5.7	4.6	5.5	0.0	5.5	0.0	0.0	0.0	3.6	4.9	4.1	7.4	0.0	8.0
	11 13 Me C25	0.0	0.0	17.4	7.4	8.5	12.4	6.4	6.4	4.2	7.0	6.4	8.6	5.2	9.9	5.9	8.0	9.5	7.1	18.8	4.9	6.9	8.0	10.1	8.9	8.5
	5 Me C25	2.7	2.6	2.6	2.5	1.7	3.9	2.6	3.3	2.8	2.2	3.2	1.5	2.6	2.5	3.2	2.9	1.9	2.7	4.8	2.1	2.4	2.7	2.7	2.8	2.6
	Heptacosene	0.0			5.9	0.0	0.0	8.2	9.2	4.1	4.9	4.1	4.5	3.9	8.1	9.6	2.8	7.7	4.6	0.0	3.6	3.8	5.1	10.7	2.9	5.1

0
0-20
20-40
40-60
60-80
80-100
100+

Figure D.6: Data sheet showing the raw substrate data for the tetradec-9-enoic acid-9,10-d_2 fatty acid experiment.

		Sub 1	Sub 2	Sub 3	Sub 4	Sub 5	Sub 6	Sub 7	Sub 8	Sub 9	Sub 10	Sub 11	Sub 12	Sub 13	Sub 14	Sub 15
Abundance of M	Tricosane	19008	15554	19408	16608	15637	15510	8989	13645	52088	6823	25880	3359	7793	12701	7074
	Pentacosene	120704	143872	99296	181440	76592	108008	61920	45040	237952	72496	328064	31440	79320	149312	61368
	Pentacosene : 2	3220	6616	3637	5996	2178	3809	1959	1560	1453	2450	5115	656	1244	853	3295
	Pentacosane	11985	6035	6799	8739	5956	4555	3405	6824	20920	2659	12022	1885	2454	6540	2932
	11 13 Me C25	14792	19120	12367	24424	9216	15054	5983	6969	45832	8829	61624	4315	14270	21312	8596
	5 Me C25	104552	122840	81488	169408	61248	88992	49664	32632	218368	59984	263488	28880	65320	103224	40168
	Heptacosene	8236	13304	7766	21184	5673	11329	4614	2059	38408	4797	44216	4236	11292	15998	6959
Abundance of M+1	Tricosane	4211	4115	5042	5557	3297	4078	2062	3461	10347	2265	5957	746	2146	3690	1747
	Pentacosene	31504	36768	27896	48168	21176	29744	18696	12388	64072	19000	91584	9983	22400	39464	15509
	Pentacosene : 2	1276	2028	999	1532	500	1448	591	288	527	598	2282	197	485	253	1510
	Pentacosane	3007	770	2234	2196	1663	1519	952	1409	6297	1362	3538	594	717	1578	1460
	11 13 Me C25	6034	12756	7922	12497	4805	10204	4365	3536	25352	5643	32432	2210	8395	12083	4297
	5 Me C25	22576	27384	18480	32320	13027	19880	10264	6566	44064	13862	57680	6354	13613	22768	10271
	Heptacosene	2551	4777	1917	9187	1690	3974	1284	704	11449	1713	11392	1100	3488	5509	2348
Abundance of M+2	Tricosane	481	415	1103	692	308	653	441	499	999	280	1067		186	629	198
	Pentacosene	6546	6978	3547	9403	2674	4124	3803	2161	10630	3743	11958	1434	4448	6547	2901
	Pentacosene : 2	235	372		829		167				211	195				253
	Pentacosane	415	274	517	620	219	187	211	314	641		419			185	
	11 13 Me C25	819	1144	996	1773	884	1006	744	254	3691	466	3302	706	819	1352	1704
	5 Me C25	1707	2903	1620	4591	1758	2514	1807	1055	5222	1449	5386	690	1927	3197	1052
	Heptacosene	551	748	365	870	409	947			2304	158	1659	280	244	1376	356
Percentage of M+1	Tricosane	22.2	26.5	26.0	33.5	21.1	26.3	22.9	25.4	19.9	33.2	23.0	22.2	27.5	29.1	24.7
	Pentacosene	26.1	25.6	28.1	26.5	27.6	27.5	30.2	27.5	26.9	26.2	27.9	31.8	28.2	26.4	25.3
	Pentacosene : 2	39.6	30.7	27.5	25.6	23.0	38.0	30.2	18.5	36.3	24.4	44.6	30.0	39.0	29.7	45.8
	Pentacosane	25.1	12.8	32.9	25.1	27.9	33.3	28.0	20.6	30.1	51.2	29.4	31.5	29.2	24.1	49.8
	11 & 13 Me C25	40.8	66.7	64.1	51.2	52.1	67.8	73.0	50.7	55.3	63.9	52.6	51.2	58.8	56.7	50.0
	5 Me C25	21.6	22.3	22.7	19.1	21.3	22.3	20.7	20.1	20.2	23.1	21.9	22.0	20.8	22.1	25.6
	Heptacosene	31.0	35.9	24.7	43.4	29.8	35.1	27.8	34.2	29.8	35.7	25.8	26.0	30.9	34.4	33.7
Percentage of M+2	Tricosane	2.5	2.7	5.7	4.2	2.0	4.2	4.9	3.7	1.9	4.1	4.1	0.0	2.4	5.0	2.8
	Pentacosene	5.4	4.9	3.6	5.2	3.5	3.8	6.1	4.8	4.5	5.2	3.6	4.6	5.6	4.4	4.7
	Pentacosene : 2	7.3	5.6	0.0	13.8	0.0	4.4	0.0	0.0	0.0	8.6	3.8	0.0	0.0	0.0	7.7
	Pentacosane	3.5	4.5	7.6	7.1	3.7	4.1	6.2	4.6	3.1	0.0	3.5	0.0	0.0	2.8	0.0
	11 13 Me C25	5.5	6.0	8.1	7.3	9.6	6.7	12.4	3.6	8.1	5.3	5.4	16.4	5.7	6.3	19.8
	5 Me C25	1.6	2.4	2.0	2.7	2.9	2.8	3.6	3.2	2.4	2.4	2.0	2.4	3.0	3.1	2.6
	Heptacosene	6.7	5.6	4.7	4.1	7.2	8.4	0.0	0.0	6.0	3.3	3.8	6.6	2.2	8.6	5.1

0
0-20
20-40
40-60
60-80
80-100
100+

Figure D.7: Data sheet showing page one of the raw substrate data for the hexadec-5-enoic acid-5,6-d_2 fatty acid experiment.

		Sub 16	Sub 17	Sub 18	Sub 19	Sub 20	Sub 21	Sub 22	Sub 23	Sub 24	Sub 25	Sub 26	Sub 27	Sub 28	Sub 29	Sub 30
Abundance of M	Tricosane	5481	25968	20224	18352	17352	26344	24064	20336	56608	29128	8689	47960	3383	24576	20704
	Pentacosene	53568	272192	208000	194240	183488	379456	208704	225664	433792	255424	117312	378112	33440	301440	191360
	Pentacosene : 2	2330	6670	2088	2057	6504	2556	1862	1794	4640	1461	1039	3759	1473	3364	3388
	Pentacosane	2536	10878	8236	8431	8551	12185	10545	8101	24712	11568	3943	19584	2299	8965	9653
	11 13 Me C25	8422	53336	42920	34400	34376	76040	27872	44256	63240	39696	22304	58432	4134	53264	33912
	5 Me C25	45040	217152	154944	141696	144704	291008	158208	167680	349120	171840	100072	271616	28840	212928	149120
	Heptacosene	6694	35240	27264	23448	24440	44976	28456	28784	55424	35936	14456	48952	4692	36448	23216
Abundance of M+1	Tricosane	1196	6719	5162	4623	4312	5020	4171	6717	12534	9283	1575	10306	908	6432	5645
	Pentacosene	13788	75264	60512	50208	48496	101200	55112	61976	116632	69080	33608	99392	8466	76288	53024
	Pentacosene : 2	560	2054	745	732	2362	1100	627	495	1587	367	444	1099	387	999	1461
	Pentacosane	806	2862	1581	2556	2592	4278	4623	1726	6470	4645	728	4689	753	2444	1559
	11 13 Me C25	3886	27280	21400	19328	17120	37232	15328	22888	33736	19768	9718	28488	2709	28552	20344
	5 Me C25	7468	47968	33464	29768	31336	60880	34184	38904	71656	39776	20936	57176	5094	48240	33184
	Heptacosene	1565	8185	7639	6267	7697	13358	8890	9012	14261	9264	4015	14127	1734	11544	7703
Abundance of M+2	Tricosane	372	699	880	760	282	4986	630	1018	1528	920	485	1504		738	621
	Pentacosene	2813	9647	7149	7435	8471	16157	8595	6469	20760	10494	6701	15303	1279	11218	6677
	Pentacosene : 2	208		282	163	231			159	327			318			
	Pentacosane		359		282		157	582	1045	1171	430	295	500	168	280	622
	11 13 Me C25	495	3339	3180	2348	2475	5245	1388	2187	3591	3319	1471	5225	425	3963	2149
	5 Me C25	978	4305	3644	2024	3490	6876	3435	633	7400	4270	2141	5387	1033	4924	4039
	Heptacosene		1600	1204	1016	1441	2050	1322	1325	3387	1214	836	2568	598	1617	658
Percentage of M+1	Tricosane	21.8	25.9	25.5	25.2	24.9	19.1	17.3	33.0	22.1	31.9	18.1	21.5	26.8	26.2	27.3
	Pentacosene	25.7	27.7	29.1	25.8	26.4	26.7	26.4	27.5	26.9	27.0	28.6	26.3	25.3	25.3	27.7
	Pentacosene : 2	24.0	30.8	35.7	35.6	36.3	43.0	33.7	27.6	34.2	25.1	42.7	29.2	26.3	29.7	43.1
	Pentacosane	31.8	26.3	19.2	30.3	30.3	35.1	43.8	21.3	26.2	40.2	18.5	23.9	32.8	27.3	16.2
	11 & 13 Me C25	46.1	51.1	49.9	56.2	49.8	49.0	55.0	51.7	53.3	49.8	43.6	48.8	65.5	53.6	60.0
	5 Me C25	16.6	22.1	21.6	21.0	21.7	20.9	21.6	23.2	20.5	23.1	20.9	21.1	17.7	22.7	22.3
	Heptacosene	23.4	23.2	28.0	26.7	31.5	29.7	31.2	31.3	25.7	25.8	27.8	28.9	37.0	31.7	33.2
Percentage of M+2	Tricosane	6.8	2.7	4.4	4.1	1.6	18.9	2.6	5.0	2.7	3.2	5.6	3.1	0.0	3.0	3.0
	Pentacosene	5.3	3.5	3.4	3.8	4.6	4.3	4.1	2.9	4.8	4.1	5.7	4.0	3.8	3.7	3.5
	Pentacosene : 2	8.9	0.0	13.5	7.9	3.6	0.0	0.0	8.9	7.0	0.0	0.0	8.5	0.0	0.0	0.0
	Pentacosane	0.0	3.3	0.0	3.3	0.0	1.3	5.5	12.9	4.7	3.7	7.5	2.6	7.3	3.1	6.4
	11 13 Me C25	5.9	6.3	7.4	6.8	7.2	6.9	5.0	4.9	5.7	8.4	6.6	8.9	10.3	7.4	6.3
	5 Me C25	2.2	2.0	2.4	1.4	2.4	2.4	2.2	0.4	2.1	2.5	2.1	2.0	3.6	2.3	2.7
	Heptacosene	0.0	4.5	4.4	4.3	5.9	4.6	4.6	4.6	6.1	3.4	5.8	5.2	12.7	4.4	2.8

0
0-20
20-40
40-60
60-80
80-100
100+

Figure D.8: Data sheet showing page two of the raw substrate data for the hexadec-5-enoic acid-5,6-d_2 fatty acid experiment.

		Sub 1	Sub 2	Sub 3	Sub 4	Sub 5	Sub 6	Sub 7	Sub 8	Sub 9	Sub 10	Sub 11	Sub 12	Sub 13	Sub 14	Sub 15	Sub 16	Sub 17	Sub 18	Sub 19	Sub 20	Sub 21
Abundance of M	Tricosane	8937		10868	6789	7277	2373	6974	6336	19752	5462	1038	10300	5474	4393		7721	13132	3038	15590		
	Pentacosene	76968		709	56968	63016	22752	60696	49008	142208	29072	14860	142144	39024	45392		63184	90568	51632	138176		
	Pentacosene : 2	2237		319	1128	1612	1078	1485	4323	825	815	304	1950	2206	1972		1746	2973	900	2597		
	Pentacosane	4172		4558	4197	3782	1560	2446	2899	7338	2928	1120	5961	3179	1718		4676	5011	2331	10141		
	11 13 Me C25	16060		14678	10173	11109	5226	14019	10547	33560	5540	1953	31328	4058	4821		10859	13060	7680	21544		
	5 Me C25	71984		61216	49208	63288	20160	7846	45936	141952	25832	9389	122608	21752	39536		58144	73960	45448	115928		
	Heptacosene	9636		8777	5608	5498	1946	7383	5592	17640	2870	1294	14407	2265	3052		6647	7527	4361	17304		
Abundance of M+1	Tricosane	2049		2235	2256	1977	569	1956	1738	3636	1109		2755	1385	1634		2595	3230	844	3928		
	Pentacosene	17808		190	16094	16323	6705	14911	12618	42120	7955	3105	37864	10631	12296		17832	24480	13613	37432		
	Pentacosene : 2	890		215	239	430	530	634	1224	278	244	185	687	1312	668		584	470	162	631		
	Pentacosane	1588		1696	1068	1010	393	942	757	3302	0		1663	1259	1186		1028	1574	605	2495		
	11 13 Me C25	7843		6504	5540	7148	1951	8681	7405	16099	2005	1018	15706	2116	2676		5426	8159	3458	10617		
	5 Me C25	15658		12306	11150	13622	4825	1935	11297	30800	5403	2905	26704	4682	7256		12521	16229	10481	24864		
	Heptacosene	2283		2022	2207	2002	1194	2276	1413	6714	708	389	4113	387	1151		1984	1648	1676	3606		
Abundance of M+2	Tricosane			364		206		414	182	682			234	509	329		186	221		633		
	Pentacosene	3037			1879	296	752	2091	1921	6579	1289	457	4403	1140	2339		1553	2928	2670	4307		
	Pentacosene : 2					169			225													
	Pentacosane	245				204		181		238			282	184			893			374		
	11 13 Me C25	966		773	639	932	527	1247	1144	2620		213	1999	262			378	1006	993	2009		
	5 Me C25	2307		1295	1257	1936	866	158	1094	3118	822	824	2611	697	1144		1250	1844	1031	2582		
	Heptacosene	475		640	233	212	228	615		1351			702		369		695		446	689		
Percentage of M+1	Tricosane	22.9		20.6	33.2	27.2	24.0	28.0	27.4	18.4	20.3	0.0	26.7	25.3	37.2		33.6	24.6	27.8	25.2		
	Pentacosene	23.1		26.8	28.3	25.9	29.5	24.6	25.7	29.6	27.4	20.9	26.6	27.2	27.1		28.2	27.0	26.4	27.1		
	Pentacosene : 2	39.8		67.4	21.2	26.7	49.2	42.7	28.3	33.7	29.9	60.9	35.2	59.5	33.9		33.4	15.8	18.0	24.3		
	Pentacosane	38.1		37.2	25.4	26.7	25.2	38.5	26.1	45.0	0.0	0.0	27.9	39.6	69.0		22.0	31.4	26.0	24.6		
	11 & 13 Me C25	48.8		44.3	54.5	64.3	37.3	61.9	70.2	48.0	36.2	52.1	50.1	52.1	55.5		50.0	62.5	45.0	49.3		
	5 Me C25	21.8		20.1	22.7	21.5	23.9	24.7	24.6	21.7	20.9	30.9	21.8	21.5	18.4		21.5	21.9	23.1	21.4		
	Heptacosene	23.7		23.0	39.4	36.4	61.4	30.8	25.3	38.1	24.7	30.1	28.5	17.1	37.7		29.8	21.9	38.4	20.8		
Percentage of M+2	Tricosane	0.0		3.3	0.0	2.8	0.0	5.9	2.9	3.5	0.0	0.0	2.3	9.3	7.5		2.4	1.7	0.0	4.1		
	Pentacosene	3.9		0.0	3.3	0.5	3.3	3.4	3.9	4.6	4.4	3.1	3.1	2.9	5.2		2.5	3.2	5.2	3.1		
	Pentacosene : 2	0.0		0.0	0.0	10.5	0.0	0.0	5.2	0.0	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0		
	Pentacosane	5.9		0.0	0.0	5.4	0.0	7.4	0.0	3.2	0.0	0.0	4.7	5.8	0.0		19.1	0.0	0.0	3.7		
	11 13 Me C25	6.0		5.3	6.3	8.4	10.1	8.9	10.8	7.8	0.0	10.9	6.4	6.5	0.0		3.5	7.7	12.9	9.3		
	5 Me C25	3.2		2.1	2.6	3.1	4.3	2.0	2.4	2.2	3.2	8.8	2.1	3.2	2.9		2.1	2.5	2.3	2.2		
	Heptacosene	4.9		7.3	4.2	3.9	11.7	8.3	0.0	7.7	0.0	0.0	4.9	0.0	12.1		10.5	0.0	10.2	4.0		

0
0-20
20-40
40-60
60-80
80-100
100+

Figure D.9: Data sheet showing page one of the raw substrate data for the hexadec-6-enoic acid-6,7-d_2 fatty acid experiment.

		Sub 22	Sub 23	Sub 24	Sub 25	Sub 26	Sub 27	Sub 28	Sub 29	Sub 30	Sub 31	Sub 32	Sub 33	Sub 34	Sub 35	Sub 36	Sub 37	Sub 38	Sub 39	Sub 40	Sub 41	Sub 42
Abundance of M	Tricosane				11500	11099	4884	15947	12324	2132	29720	21768	17848	39768	12500	14940	17344	19960	30568	28312	39360	24944
	Pentacosene				124960	107360	46728	152064	143232	19664	204864	274816	139008	504064	90344	123440	184128	199488	323712	312320	91592	217664
	Pentacosene : 2				690	1107	1087	1009	900	945	1738	2515	861	2709	1040	1090	1853	1698	1635	2830	4087	2124
	Pentacosane				5798	5015	3412	4858	5732	2347	17688	13446	7953	16059	9065	9205	7708	7034	11068	16728	16680	11031
	11 13 Me C25				17896	24488	4173	27832	25192	2293	30704	52736	22528	89448	11768	20888	27280	38064	65008	49152	74280	41712
	5 Me C25				117552	89168	34928	129896	121968	14650	174144	233920	126416	423808	77544	114632	147072	152448	281344	257152	295040	191552
	Heptacosene				14680	14434	4568	20216	18272	1613	28040	33552	18736	63192	11130	16936	23440	25152	41872	45152	48880	25544
Abundance of M+1	Tricosane				4340	3237	933	3822	2915	437	7694	6095	7000	10204	3358	3773	6161	4604	6038	8076	11426	6558
	Pentacosene				35792	27040	11433	43032	41472	7328	53336	74928	34976	138304	23544	32992	44384	56272	88064	78384	11281	59048
	Pentacosene : 2				363	346	396	711	608	177	726	840	258	1746	258	300	550	674	649	1162	1047	686
	Pentacosane				2328	1680	935	1845	2487	1106	3910	2428	2344	5607	2502	2414	2015	1800	3324	4875	4990	2456
	11 13 Me C25				9699	13866	2153	15077	12620	1004	15396	28328	8771	51416	6878	10491	15501	20296	29008	21936	34840	23128
	5 Me C25				23888	20216	9090	28752	27640	4332	37808	51512	28560	89592	15031	23784	27968	30568	60696	56576	56024	36800
	Heptacosene				4490	3585	1349	5161	4166	750	8422	9552	4972	19584	3218	5125	6477	7076	12366	14081	12761	8746
Abundance of M+2	Tricosane				853	768		454	356		508	1052	246	1160	274	469	1168	642	986	897	951	923
	Pentacosene				3865	4046	1221	5092	6381	1150	8687	8867	5145	16880	3449	4189	5968	8077	11889	12190	1391	8488
	Pentacosene : 2							180			292											
	Pentacosane				428	284		193			1024	778	633	963	235	522	446	236	713	764	1211	186
	11 13 Me C25				1226	1803		2256	1216		1130	3629	2189	3535	1092	1490	3090	2112	5480	4408	4549	4490
	5 Me C25				2715	2019		3184	2745	667	4290	5185	3757	7766	1664	3295	3871	3057	6293	6417	8339	4339
	Heptacosene				554	468	357	1024	514		1274	1187	1385	2773	488	511	455	1632	1675	2044	3136	1016
Percentage of M+1	Tricosane				37.7	29.2	19.1	24.0	23.7	20.5	25.9	28.0	39.2	25.7	26.9	25.3	35.5	23.1	19.8	28.5	29.0	26.3
	Pentacosene				28.6	25.2	24.5	28.3	29.0	37.3	26.0	27.3	25.2	27.4	26.1	26.7	24.1	28.2	27.2	25.1	12.3	27.1
	Pentacosene : 2				52.6	31.3	36.4	70.5	67.6	18.7	41.8	33.4	30.0	64.5	24.8	27.5	29.7	39.7	39.7	41.1	25.6	32.3
	Pentacosane				40.2	33.5	27.4	38.0	43.4	47.1	22.1	18.1	29.5	34.9	27.6	26.2	26.1	25.6	30.0	29.1	29.9	22.3
	11 & 13 Me C25				54.2	56.6	51.6	54.2	50.1	43.8	50.1	53.7	38.9	57.5	58.4	50.2	56.8	53.3	44.6	44.6	46.9	55.4
	5 Me C25				20.3	22.7	26.0	22.1	22.7	29.6	21.7	22.0	22.6	21.1	19.4	20.7	19.0	20.1	21.6	22.0	19.0	19.2
	Heptacosene				30.6	24.8	29.5	25.5	22.8	46.5	30.0	28.5	26.5	31.0	28.9	30.3	27.6	28.1	29.5	31.2	26.1	34.2
Percentage of M+2	Tricosane				7.4	6.9	0.0	2.8	2.9	0.0	1.7	4.8	1.4	2.9	2.2	3.1	6.7	3.2	3.2	3.2	2.4	3.7
	Pentacosene				3.1	3.8	2.6	3.3	4.5	5.8	4.2	3.2	3.7	3.3	3.8	3.4	3.2	4.0	3.7	3.9	1.5	3.9
	Pentacosene : 2				0.0	0.0	0.0	17.8	0.0	0.0	16.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Pentacosane				7.4	5.7	0.0	4.0	0.0	0.0	5.8	5.8	8.0	6.0	2.6	5.7	5.8	3.4	6.4	4.6	7.3	1.7
	11 13 Me C25				6.9	7.4	0.0	8.1	4.8	0.0	3.7	6.9	9.7	4.0	9.3	7.1	11.3	5.5	8.4	9.0	6.1	10.8
	5 Me C25				2.3	2.3	0.0	2.5	2.3	4.6	2.5	2.2	3.0	1.8	2.1	2.9	2.6	2.0	2.2	2.5	2.8	2.3
	Heptacosene				3.8	3.2	7.8	5.1	2.8	0.0	4.5	3.5	7.4	4.4	4.4	3.0	1.9	6.5	4.0	4.5	6.4	4.0

0
0-20
20-40
40-60
60-80
80-100
100+

Figure D.10: Data sheet showing page two of the raw substrate data for the hexadec-6-enoic acid-6,7-d_2 fatty acid experiment.

		Sub 1	Sub 2	Sub 3	Sub 4	Sub 5	Sub 6	Sub 7	Sub 8	Sub 9	Sub 10	Sub 11	Sub 12	Sub 13	Sub 14	Sub 15	Sub 16	Sub 17	Sub 18	Sub 19
Abundance of M	Tricosane	3789	4288	9436	14895	16129	14126	36424	10565	9477	10642	6498	9954	7576	12631	8780	8805	20760	26208	9031
	Pentacosene	33256	72384	90928	160256	169472	79368	222912	105976	124096	157248	110456	84408	71336	171712	115464	144074	230656	264064	74328
	Pentacosene : 2	1394	2179	755	5182	1548	2152	1251	1693	2515	3008	577	1393	900	1625	1749	1835	5481	1718	627
	Pentacosane	2172	2041	6242	4346	5306	6099	17528	4844	4126	3885	2481	4220	2642	4408	3899	5530	10013	9262	4083
	11 13 Me C25	2932	16230	12529	31848	38736	5614	31560	14874	24728	25040	20296	13809	5485	27024	11935	21536	32272	54384	8103
	5 Me C25	29768	66520	83584	140480	167040	47408	184256	77664	96896	130488	83920	60304	61624	133056	69912	117776	153216	215488	50144
	Heptacosene	3514	11217	15608	23624	23768	2100	34408	14037	15492	22104	13130	9568	7928	18088	10931	22536	31640	36352	10673
Abundance of M+1	Tricosane	717	1237	3446	4069	3304	3597	9876	3663	2611	2597	2366	2752	1566	2765	2174	3159	4762	6807	2810
	Pentacosene	7868	19336	24480	44784	46936	22016	54968	28888	33336	44816	30584	20040	22096	46264	28584	36720	62112	72288	18792
	Pentacosene : 2	713	531	0	1688	180	922	506	778	983	1007	460	509	159	277	470	353	849	196	228
	Pentacosane	1150	466	1392	1618	2027	1861	5832	1466	777	1257	1330	1353	1043	477	1551	1843	2060	2430	346
	11 13 Me C25	1908	7668	7907	15667	18784	3752	16752	9448	11581	13610	11144	6025	966	14623	6690	13169	14992	27048	4997
	5 Me C25	7583	16600	20440	34312	35416	10466	40816	18112	20920	25872	17312	15764	13072	30376	13585	25544	36472	45632	10375
	Heptacosene	1355	3328	3747	5241	7910	1210	9856	4601	3578	6349	4572	3348	4035	6747	4271	6154	10191	11027	2375
Abundance of M+2	Tricosane	277	294	215	229	689	537	1664	531	366	537	221	341	212	1014	208	315		1024	
	Pentacosene	1700	2014	3722	5929	4875	1913	7722	3533	5567	5090	3244	2407	3159	5288	2564	6177	10417	8323	2268
	Pentacosene : 2	0	155	0	349	0	0	0	191	0	0									
	Pentacosane	164	219	1065	216	221	216	942	188	0	590		219		337	261		845	236	247
	11 13 Me C25	328	1365	855	2338	3084	697	3084	1185	1928	2027	748	1739		1465	740	999	2369	3405	394
	5 Me C25	475	2623	2308	4292	3610	10466	3726	2095	2152	2636	1930	1179	1682	4027	2098	2655	3702	4981	1310
	Heptacosene	804	769	336	442	2505	300	1697	646	1084	942	293		620	881	281	689	1741	1682	426
Percentage of M+1	Tricosane	18.9	28.8	36.5	27.3	20.5	25.5	27.1	34.7	27.6	24.4	36.4	27.6	20.7	21.9	24.8	35.9	22.9	26.0	31.1
	Pentacosene	23.7	26.7	26.9	27.9	27.7	27.7	24.7	27.3	26.9	28.5	27.7	23.7	31.0	26.9	24.8	25.5	26.9	27.4	25.3
	Pentacosene : 2	51.1	24.4	0.0	32.6	11.6	42.8	40.4	46.0	39.1	33.5	79.7	36.5	17.7	17.0	26.9	19.2	15.5	11.4	36.4
	Pentacosane	52.9	22.8	22.3	37.2	38.2	30.5	33.3	30.3	18.8	32.4	53.6	32.1	39.5	10.8	39.8	33.3	20.6	26.2	8.5
	11 & 13 Me C25	65.1	47.2	63.1	49.2	48.5	66.8	53.1	63.5	46.8	54.4	54.9	43.6	17.6	54.1	56.1	61.1	46.5	49.7	61.7
	5 Me C25	25.5	25.0	24.5	24.4	21.2	22.1	22.2	23.3	21.6	19.8	20.6	26.1	21.2	22.8	19.4	21.7	23.8	21.2	20.7
	Heptacosene	38.6	29.7	24.0	22.2	33.3	57.6	28.6	32.8	23.1	28.7	34.8	35.0	50.9	37.3	39.1	27.3	32.2	30.3	22.3
Percentage of M+2	Tricosane	7.3	6.9	2.3	1.5	4.3	3.8	4.6	5.0	3.9	5.0	3.4	3.4	2.8	8.0	2.4	3.6	0.0	3.9	0.0
	Pentacosene	5.1	2.8	4.1	3.7	2.9	2.4	3.5	3.3	4.5	3.2	2.9	2.9	4.4	3.1	2.2	4.3	4.5	3.2	3.1
	Pentacosene : 2	0.0	7.1	0.0	6.7	0.0	0.0	0.0	11.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Pentacosane	7.6	10.7	17.1	5.0	4.2	3.5	5.4	3.9	0.0	15.2	0.0	5.2	0.0	7.6	6.7	0.0	8.4	2.5	6.0
	11 13 Me C25	11.2	8.4	6.8	7.3	8.0	12.4	9.8	8.0	7.8	8.1	3.7	12.6	0.0	5.4	6.2	4.6	7.3	6.3	4.9
	5 Me C25	1.6	3.9	2.8	3.1	2.2	22.1	2.0	2.7	2.2	2.0	2.3	2.0	2.7	3.0	3.0	2.3	2.4	2.3	2.6
	Heptacosene	22.9	6.9	2.2	1.9	10.5	14.3	4.9	4.6	7.0	4.3	2.2	0.0	7.8	4.9	2.6	3.1	5.5	4.6	4.0

0
0-20
20-40
40-60
60-80
80-100
100+

Figure D.11: Data sheet showing page one of the raw substrate data for the hexadec-7-enoic acid-7,8-d_2 fatty acid experiment.

		Sub 26	Sub 27	Sub 28	Sub 29	Sub 30	Sub 31	Sub 32	Sub 33	Sub 34	Sub 35	Sub 36	Sub 37	Sub 38	Sub 39	Sub 40	Sub 41	Sub 42	Sub 43	Sub 44
Abundance of M	Tricosane	6386	12327	18496	25608	25088	6089	12206	30456	16552	8266	10122	12378	10831	13414	39448	24760	23024	11302	23464
	Pentacosene	77936	117168	342272	219328	359872	89968	129272	301888	79360	41168	27928	187008	151488	143552	536576	219584	201728	171584	173504
	Pentacosene : 2	1418	739	1990	1473	1767	1045	1575	1810	1170	803	1313	2143	2356	1249	1702	5296	1223	962	952
	Pentacosane	3577	4303	10923	9589	10289	4074	5289	10580	7233	3769	2721	5500	2992	5171	16584	9621	5081	4582	8918
	11 13 Me C25	12214	13865	50168	37640	56376	9048	18344	60424	10390	5370	3244	29960	17992	24152	63328	27168	29592	24600	20824
	5 Me C25	52072	76680	242752	148992	264000	60496	106976	222592	54096	32728	18544	123624	93992	83888	288832	142912	138240	116632	109344
	Heptacosene	9678	13714	43928	25904	42184	14159	18064	33584	8752	4403	2955	28000	17336	16984	73064	28488	26912	22696	23560
Abundance of M+1	Tricosane	2016	3022	4487	5425	8095	1553	2804	8113	4887	2100	2922	5044	2725	3843	8313	5861	6718	2941	6630
	Pentacosene	21368	30280	82688	60424	89280	23320	34288	81568	20272	10293	6777	45256	40656	41368	139456	48960	53656	44096	38584
	Pentacosene : 2	366	186	456	461	926	592	488	362	232	340	172	775	1434	523	441	1372	365	839	384
	Pentacosane	993	2018	1513	2950	2483	1017	1102	3242	2407	352	929	1059	1337	1256	4766	2436	2300	1102	1746
	11 13 Me C25	6907	9001	27616	18152	30176	5598	6441	27976	5490	2427	1292	15834	9004	11493	36520	16536	13709	13374	12138
	5 Me C25	12387	15237	56840	37704	56080	13726	22928	50808	12452	8023	3792	24992	21264	19328	67288	27960	28448	22760	23192
	Heptacosene	3490	3631	11230	8534	11302	3372	4934	7976	3072	1491	620	7086	7656	4802	21296	7763	8955	5611	6410
Abundance of M+2	Tricosane		548		693	876			810	197	337	167	430	698	209	1688	588	536	801	917
	Pentacosene	3775	3915	10069	6815	11439	2873	3941	12354	1839	755	1788	5585	4494	5286	17504	7895	6733	6321	6820
	Pentacosene : 2																			
	Pentacosane		467	445	162	707	180		417		264	287	341	274	244	653	413	210		553
	11 13 Me C25	1110	884	3184	2206	5252		1748	4804	534	355		2228	1984	1321	5353	948	1857	1415	1039
	5 Me C25	1857	1768	6260	3601	5676	1042	1915	4664	1404		254	2618	2297	2854	6787	4003	2497	2881	4084
	Heptacosene		554	2464	1089	3402	776	634	1334	463	501		1462	1092	646	2678	1467	1843	1231	1614
Percentage of M+1	Tricosane	31.6	24.5	24.3	21.2	32.3	25.5	23.0	26.6	29.5	25.4	28.9	40.7	25.2	28.6	21.1	23.7	29.2	26.0	28.3
	Pentacosene	27.4	25.8	24.2	27.5	24.8	25.9	26.5	27.0	25.5	25.0	24.3	24.2	26.8	28.8	26.0	22.3	26.6	25.7	22.2
	Pentacosene : 2	25.8	25.2	22.9	31.3	52.4	56.7	31.0	20.0	19.8	42.3	13.1	36.2	60.9	41.9	25.9	25.9	29.8	87.2	40.3
	Pentacosane	27.8	46.9	13.9	30.8	24.1	25.0	20.8	30.6	33.3	9.3	34.1	19.3	44.7	24.3	28.7	25.3	45.3	24.1	19.6
	11 & 13 Me C25	56.5	64.9	55.0	48.2	53.5	61.9	35.1	46.3	52.8	45.2	39.8	52.9	50.0	47.6	57.7	60.9	46.3	54.4	58.3
	5 Me C25	23.8	19.9	23.4	25.3	21.2	22.7	21.4	22.8	23.0	24.5	20.4	20.2	22.6	23.0	23.3	19.6	20.6	19.5	21.2
	Heptacosene	36.1	26.5	25.6	32.9	26.8	23.8	27.3	23.7	35.1	33.9	21.0	25.3	44.2	28.3	29.1	27.3	33.3	24.7	27.2
Percentage of M+2	Tricosane	0.0	4.4	0.0	2.7	3.5	0.0	0.0	2.7	1.2	4.1	1.6	3.5	6.4	1.6	4.3	2.4	2.3	7.1	3.9
	Pentacosene	4.8	3.3	2.9	3.1	3.2	3.2	3.0	4.1	2.3	1.8	6.4	3.0	3.0	3.7	3.3	3.6	3.3	3.7	3.9
	Pentacosene : 2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Pentacosane	0.0	10.9	4.1	1.7	6.9	4.4	0.0	3.9	0.0	7.0	10.5	6.2	9.2	4.7	3.9	4.3	4.1	0.0	6.2
	11 13 Me C25	9.1	6.4	6.3	5.9	9.3	0.0	9.5	8.0	5.1	6.6	0.0	7.4	11.0	5.5	8.5	3.5	6.3	5.8	5.0
	5 Me C25	3.6	2.3	2.6	2.4	2.2	1.7	1.8	2.1	2.6	0.0	1.4	2.1	2.4	3.4	2.3	2.8	1.8	2.5	3.7
	Heptacosene	0.0	4.0	5.6	4.2	8.1	5.5	3.5	4.0	5.3	11.4	0.0	5.2	6.3	3.8	3.7	5.1	6.8	5.4	6.9

0
0-20
20-40
40-60
60-80
80-100
100+

Figure D.12: Data sheet showing page two of the raw substrate data for the hexadec-7-enoic acid-7,8-d_2 fatty acid experiment.

		Sub 1	Sub 2	Sub 3	Sub 4	Sub 5	Sub 6	Sub 7	Sub 8	Sub 9	Sub 10	Sub 11	Sub 12	Sub 13	Sub 14	Sub 15	Sub 16	Sub 17	Sub 18	Sub 19	Sub 20	Sub 21
Abundance of M	Tricosane	49248	9990	38408	21168	74504	31256	73032	57480	40672	28112	74960	68848	66624	40304	17872	60408	69792	62736	50536	77064	82208
	Pentacosene	280000	145152	394624	207104	497792	182976	505088	462656	271552	307072	543424	345664	330944	258432	183296	448960	523840	454720	375744	404736	504384
	Pentacosene : 2	22488	10401	16260	5360	23272	4045	17752	22128	7232	6342	22400	11045	8289	5772	6217	11785	21792	13752	11115	7630	19840
	Pentacosane	8493	6852	16440	9973	32584	11472	26048	11962	12098	11922	25616	28776	21472	11220	11971	28880	19704	36712	24424	33960	38560
	11 13 Me C25	30408	9377	32000	8993	33680	12292	69040	79608	15384	19760	94648	30096	20520	17024	11606	36576	118048	45984	38248	21920	61888
	5 Me C25	1007060	48872	201664	62416	279424	72856	252416	195968	121168	141440	284928	175680	138944	58168	81760	211968	264128	263040	202752	176768	291392
	Heptacosene	20224	2010	49024	12254	71552	14872	81264	72672	29176	40776	80984	45424	40152	31744	17016	69976	94680	66944	67312	51544	76792
Abundance of M+1	Tricosane	11594	5099	11517	8235	17776	6649	19440	16278	9502	7183	15821	17144	14955	8449	6400	11972	21016	14348	12330	14046	26312
	Pentacosene	70032	34264	102352	57248	126256	41248	137792	124752	78424	76616	20312	97072	98256	68296	49032	123120	142912	117760	107608	110280	132032
	Pentacosene : 2	4369	3387	4779	2747	4926	2011	6166	5750	2174	2854	3993	2572	3233	1667	2373	2217	7073	4928	3112	1858	7518
	Pentacosane	2454	2692	2590	1817	7759	3727	7177	5600	4506	2822	6891	7905	6401	2399	4094	7704	5912	9884	6505	9915	9004
	11 13 Me C25	14730	6955	22432	6883	25832	5511	36304	44984	9883	9235	42888	24304	15434	10394	6285	17680	65168	24752	24000	12500	38120
	5 Me C25	27424	15774	40984	15801	57384	18136	56256	45840	23784	34632	59952	36816	30712	16760	15570	44168	51152	55640	49768	40048	62376
	Heptacosene	5366	1001	16984	2912	27288	10181	24032	22824	11230	12404	25088	14200	12541	7261	4857	21832	28040	22944	18752	12655	28976
Abundance of M+2	Tricosane	724	180	823	188	1946	1341	3352	2141	1168	502	2245	701	2044	1474	1236	1317	2472	2595	2291	15393	2428
	Pentacosene	9610	5837	13705	7863	18576	6266	20112	15692	10961	14544	1232	9820	13181	7017	7466	15487	16026	14842	15169	10859	12330
	Pentacosene : 2	1442	894	908	1141	1123	201	1099	312	583	475	574	351					1420	327	727	631	784
	Pentacosane	1346	447	1057	296	518	213	981		528	898	661	696	512			646	530	922	739	2096	1794
	11 13 Me C25	2314	1109	2635	582	2515	2041	5369	4734	527	2039	9307	1707	1063	1112	813	4168	9234	4934	3138	1008	5842
	5 Me C25	3279	1328	6402	1077	5878	1491	8021	4829	2841	4747	11211	5262	4461	936	2382	5075	4757	5386	4914	3456	9998
	Heptacosene	663		2847	452	2561	1603	2193	2734	970	1895	3736	1116	728	2402	941	2376	4280	3098	3819	1556	3093
Percentage of M+1	Tricosane	23.5	51.0	30.0	38.9	23.9	21.3	26.6	28.3	23.4	25.6	21.1	24.9	22.4	21.0	35.8	19.8	30.1	22.9	24.4	18.2	32.0
	Pentacosene	25.0	23.6	25.9	27.6	25.4	22.5	27.3	27.0	28.9	25.0	3.7	28.1	29.7	26.4	26.8	27.4	27.3	25.9	28.6	27.2	26.2
	Pentacosene : 2	19.4	32.6	29.4	51.3	21.2	49.7	34.7	26.0	30.1	45.0	17.8	23.3	39.0	28.9	38.2	18.8	32.5	35.8	28.0	24.4	37.9
	Pentacosane	28.9	39.3	15.8	18.2	23.8	32.5	27.6	46.8	37.2	23.7	26.9	27.5	29.8	21.4	34.2	26.7	30.0	26.9	26.6	29.2	23.4
	11 & 13 Me C25	48.4	74.2	70.1	76.5	76.7	44.8	52.6	56.5	64.2	46.7	45.3	80.8	75.2	61.1	54.2	48.3	55.2	53.8	62.7	57.0	61.6
	5 Me C25	2.7	32.3	20.3	25.3	20.5	24.9	22.3	23.4	19.6	24.5	21.0	21.0	22.1	28.8	19.0	20.8	19.4	21.2	24.5	22.7	21.4
	Heptacosene	26.5	49.8	34.6	23.8	38.1	68.5	29.6	31.4	38.5	30.4	31.0	31.3	31.2	22.9	28.5	31.2	29.6	34.3	27.9	24.6	37.7
Percentage of M+2	Tricosane	1.5	1.8	2.1	0.9	2.6	4.3	4.6	3.7	2.9	1.8	3.0	1.0	3.1	3.7	6.9	2.2	3.5	4.1	4.5	20.0	3.0
	Pentacosene	3.4	4.0	3.5	3.8	3.7	3.4	4.0	3.4	4.0	4.7	0.2	2.8	4.0	2.7	4.1	3.4	3.1	3.3	4.0	2.7	2.4
	Pentacosene : 2	6.4	8.6	5.6	21.3	4.8	5.0	6.2	1.4	8.1	7.5	2.6	3.2	0.0	0.0	0.0	0.0	6.5	2.4	6.5	8.3	4.0
	Pentacosane	15.8	6.5	6.4	3.0	1.6	1.9	3.8	0.0	4.4	7.5	2.6	2.4	2.4	0.0	0.0	2.2	2.7	2.5	3.0	6.2	4.7
	11 13 Me C25	7.6	11.8	8.2	6.5	7.5	16.6	7.8	5.9	3.4	10.3	9.8	5.7	5.2	6.5	7.0	11.4	7.8	10.7	8.2	4.6	9.4
	5 Me C25	0.3	2.7	3.2	1.7	2.1	2.0	3.2	2.5	2.3	3.4	3.9	3.0	3.2	1.6	2.9	2.4	1.8	2.0	2.4	2.0	3.4
	Heptacosene	3.3	0.0	5.8	3.7	3.6	10.8	2.7	3.8	3.3	4.6	4.6	2.5	1.8	7.6	5.5	3.4	4.5	4.6	5.7	3.0	4.0

0
0-20
20-40
40-60
60-80
80-100
100+

Figure D.13: Data sheet showing page one of the raw substrate data for the hexadec-9-enoic acid-9,10-d_2 fatty acid experiment.

		Sub 22	Sub 23	Sub 24	Sub 25	Sub 26	Sub 27	Sub 28	Sub 29	Sub 30	Sub 31	Sub 32	Sub 33	Sub 34	Sub 35	Sub 36	Sub 37	Sub 38	Sub 39	Sub 40	Sub 41	Sub 42
Abundance of M	Tricosane	50256	66480	83128	56088	136576	69232	70752	62048	98168	64712	65584	64208			101776	53320		74352	85544	59416	81176
	Pentacosene	360192	498624	500032	8908	763584	608256	582400	638208	529856	388416	541696	460160			606720	484544		627904	553216	338432	505792
	Pentacosene : 2	9152	12747	12726	21488	15186	5785	12916	7002	9385	8792	8053	6072			6236	10747		5990	15358	6411	13906
	Pentacosane	20688	30448	32616	28240	48976	20672	32376	33200	36328	27832	16204	31696			28688	33088		24208	31616	23376	40712
	11 13 Me C25	69336	38600	49352	28000	113928	202688	52168	86448	44648	39456	154112	53512			83320	31784		153728	80496	37952	36744
	5 Me C25	184000	241984	257024	181888	405632	300288	335616	397888	227072	239744	307712	220864			365760	210048		377920	305152	122544	241728
	Heptacosene	64832	73208	81288	45528	128088	137920	95280	113392	87304	55704	106112	67712			105016	78008		116632	105328	47912	73544
Abundance of M+1	Tricosane	15743	17672	23016	13795	35368	13250	21000	19120	20224	12267	18552	20096			25944	13496		17776	25136	15596	16624
	Pentacosene	104584	128328	139200	1684	201664	151808	163008	170944	142592	111080	155200	177184			148480	130680		165376	148992	89016	133184
	Pentacosene : 2	2095	3386	4448	8922	3649	2003	2334	3696	1929	2844	1355	2746			798	1651		2061	3161	2418	4178
	Pentacosane	4314	6708	10002	15838	12220	6199	7978	5528	11133	8521	4954	9646			9541	12005		5894	6334	7023	10277
	11 13 Me C25	33624	29560	28200	13688	56760	102960	28928	46784	28192	19640	81808	33280			38064	14415		89624	48048	24536	20688
	5 Me C25	34472	63272	49448	42248	97048	69880	69560	91944	48840	52984	28552	44776			73656	39840		84144	73776	22704	57080
	Heptacosene	16113	21296	26208	19464	39112	43208	26000	37912	23144	16241	2852	19456			29952	17800		33952	33112	12560	23728
Abundance of M+2	Tricosane	563	3227	2804	1915	2728	2862	927	2709	3677	1225	1348	3500			2251	1068		2824	3666	2494	2513
	Pentacosene	13603	14518	17192	858	29328	24664	21472	20728	20296	18936	23280	19104			16584	16416		20176	14659	14492	16223
	Pentacosene : 2		200	725	450	292	411	376	846	581			242			342	949		377	564	454	246
	Pentacosane	1545	1213	3383	2109	1767	644	538	1333	851	529	953	441			1006	2339		734	1231	381	1869
	11 13 Me C25	4169	4776	4848	1974	8807	16147	6015	5295	4042	2182	13187	4804			4964	4770		10332	6021	3463	3343
	5 Me C25	3263	4758	6692	2967	10650	7222	9935	9151	6256	7984	5672	4161			9721	4329		6108	12103	3281	7930
	Heptacosene	1478	6232	5595	2313	5930	5369	3222	3857	4067	2918	4461	2665			4996	4458		4999	5281	2407	4643
Percentage of M+1	Tricosane	31.3	26.6	27.7	24.6	25.9	19.1	29.7	30.8	20.6	19.0	28.3	31.3			25.5	25.3		23.9	29.4	26.2	20.5
	Pentacosene	29.0	25.7	27.8	18.9	26.4	25.0	28.0	26.8	26.9	28.6	28.7	38.5			24.5	27.0		26.3	26.9	26.3	26.3
	Pentacosene : 2	22.9	26.6	35.0	41.5	24.0	34.6	18.1	52.8	20.6	32.3	16.8	45.2			12.8	15.4		34.4	20.6	37.7	30.0
	Pentacosane	20.9	22.0	30.7	56.1	25.0	30.0	24.6	16.7	30.6	30.6	30.6	30.4			33.3	36.3		24.3	20.0	30.0	25.2
	11 & 13 Me C25	48.5	76.6	57.1	48.9	49.8	50.8	55.5	54.1	63.1	49.8	53.1	62.2			45.7	45.4		58.3	59.7	64.7	56.3
	5 Me C25	18.7	26.1	19.2	23.2	23.9	23.3	20.7	23.1	21.5	22.1	9.3	20.3			20.1	19.0		22.3	24.2	18.5	23.6
	Heptacosene	24.9	29.1	32.2	42.8	30.5	31.3	27.3	33.4	26.5	29.2	2.7	28.7			28.5	22.8		29.1	31.4	26.2	32.3
Percentage of M+2	Tricosane	1.1	4.9	3.4	3.4	2.0	4.1	1.3	4.4	3.7	1.9	2.1	5.5			2.2	2.0		3.8	4.3	4.2	3.1
	Pentacosene	3.8	2.9	3.4	9.6	3.8	4.1	3.7	3.2	3.8	4.9	4.3	4.2			2.7	3.4		3.2	2.6	4.3	3.2
	Pentacosene : 2	0.0	1.6	5.7	2.1	1.9	7.1	2.9	12.1	6.2	0.0	0.0	4.0			5.5	8.8		6.3	3.7	7.1	1.8
	Pentacosane	7.5	4.0	10.4	7.5	3.6	3.1	1.7	4.0	2.3	1.9	5.9	1.4			3.5	7.1		3.0	3.9	1.6	4.6
	11 13 Me C25	6.0	12.4	9.8	7.1	7.7	8.0	11.5	6.1	9.1	5.5	8.6	9.0			6.0	15.0		6.7	7.5	9.1	9.1
	5 Me C25	1.8	2.0	2.6	1.6	2.6	2.4	3.0	2.3	2.8	3.3	1.8	1.9			2.7	2.1		1.6	4.0	2.7	3.3
	Heptacosene	2.3	8.5	6.9	5.1	4.6	3.9	3.4	3.4	4.7	5.2	4.2	3.9			4.8	5.7		4.3	5.0	5.0	6.3

0
0-20
20-40
40-60
60-80
80-100
100+

Figure D.14: Data sheet showing page two of the raw substrate data for the hexadec-9-enoic acid-9,10-d_2 fatty acid experiment.

		Sub 43	Sub 44	Sub 45	Sub 46	Sub 47	Sub 48	Sub 49	Sub 50	Sub 51	Sub 52	Sub 53	Sub 54	Sub 55	Sub 56	Sub 57	Sub 58	Sub 59	Sub 60	Sub 61	Sub 62
Abundance of M	Tricosane	64480		110784	81696	33624	66840	28744	54728	65232	42296	39384	25448	19920	64552	32656	40360	32560	60760	42464	77984
	Pentacosene	476032		682816	528640	252416	393984	253760	379392	415105	214592	188160	191104	140864	396096	271808	181568	251072	332608	266624	464576
	Pentacosene : 2	11095		8315	60840	2798	3107	5403	3770	8373	4945	3059	4340	1826	5517	8624	2300	3739	4973	4565	5661
	Pentacosane	19192		33936	24976	15457	27832	9642	19064	22856	15340	29576	11141	7955	20984	11944	21600	12423	19824	11529	29744
	11 13 Me C25	131840		106848	108736	33424	59576	37296	98672	50872	56968	9925	23160	13936	108048	80976	20728	25968	40824	98632	53096
	5 Me C25	266368		350720	263616	144768	249792	142016	252736	198976	99872	74264	83928	86064	260480	151872	103888	136256	170432	172864	229888
	Heptacosene	97808		108200	81192	39792	72224	38968	77792	78120	38136	21808	20280	20064	82680	67456	28024	33216	60352	57424	80072
Abundance of M+1	Tricosane	16560		32592	21416	11257	16872	9011	13165	18416	12113	9323	6462	5909	17136	7977	6616	7183	15317	9478	18880
	Pentacosene	130336		192896	136192	61104	99112	66776	99728	117480	64024	45440	45920	35448	110496	78128	54376	68992	90656	77728	120600
	Pentacosene : 2	5373		2029	18600	978	1388	796	915	1854	1682	357	1817	1221	980	3222	949	1288	1800	1585	1989
	Pentacosane	7982		10101	8199	3624	9140	2481	6246	6083	3567	6465	2383	2068	5850	4745	4863	5134	6424	3925	8636
	11 13 Me C25	59792		62592	61152	19648	30624	16279	47624	26888	27024	6662	10689	11115	58760	50424	13075	16432	25792	58688	34736
	5 Me C25	49232		78632	63296	28632	61200	27920	49880	43368	22032	11761	17168	17800	61304	36320	22824	30096	33664	34504	55200
	Heptacosene	29296		42968	29760	10525	22872	13367	20448	21184	10064	6270	7620	5731	32976	21712	7819	12933	16808	17352	25768
Abundance of M+2	Tricosane	1549		4584	3169	1510	1428	1177	2143	1394	2118	490	488	549	3116	700	1174	2714	2866	1598	2406
	Pentacosene	16512		20128	15717	11461	15807	6996	10855	17120	6705	6788	7641	4538	14002	7781	7515	8601	11969	10260	18952
	Pentacosene : 2				3301		476	834	681	284	286			203	803	822	201	261	555		1147
	Pentacosane			1770	1135	538	1315	338	602	440	406	450		905	818	418	570	780	951	434	1634
	11 13 Me C25	7064		6553	7076	2575	4293	2396	8881	3701	3083	634	1378	2106	7058	5210	1554	2390	2752	7436	3197
	5 Me C25	9085		7065	6709	3855	7752	3336	5288	5705	2045	2880	1805	2706	6421	3300	4090	4348	5756	3373	5685
	Heptacosene	3675		5559	4435	1250	2739	1863	5676	4911	1290	1107	1013	1166	3273	2768	1491	1319	3040	2978	4030
Percentage of M+1	Tricosane	25.7		29.4	26.2	33.5	25.2	31.3	24.1	28.2	28.6	23.7	25.4	29.7	26.5	24.4	16.4	22.1	25.2	22.3	24.2
	Pentacosene	27.4		28.3	25.8	24.2	25.2	26.3	26.3	28.3	29.8	24.1	24.0	25.2	27.9	28.7	29.9	27.5	27.3	29.2	26.0
	Pentacosene : 2	48.4		24.4	30.6	35.0	44.7	14.7	24.3	22.1	34.0	11.7	41.9	66.9	17.8	37.4	41.3	34.4	36.2	34.7	35.1
	Pentacosane	41.6		29.8	32.8	23.4	32.8	25.7	32.8	26.6	23.3	21.9	21.4	26.0	27.9	39.7	22.5	41.3	32.4	34.0	29.0
	11 & 13 Me C25	45.4		58.6	56.2	58.8	51.4	43.6	48.3	52.9	47.4	67.1	46.2	79.8	54.4	62.3	63.1	63.3	63.2	59.5	65.4
	5 Me C25	18.5		22.4	24.0	19.8	24.5	19.7	19.7	21.8	22.1	15.8	20.5	20.7	23.5	23.9	22.0	22.1	19.8	20.0	24.0
	Heptacosene	30.0		39.7	36.7	26.5	31.7	34.3	26.3	27.1	26.4	28.8	37.6	28.6	39.9	32.2	27.9	38.9	27.8	30.2	32.2
Percentage of M+2	Tricosane	2.4		4.1	3.9	4.5	2.1	4.1	3.9	2.1	5.0	1.2	1.9	2.8	4.8	2.1	2.9	8.3	4.7	3.8	3.1
	Pentacosene	3.5		2.9	3.0	4.5	4.0	2.8	2.9	4.1	3.1	3.6	4.0	3.2	3.5	2.9	4.1	3.4	3.6	3.8	4.1
	Pentacosene : 2	0.0		0.0	5.4	0.0	15.3	15.4	18.1	3.4	5.8	0.0	0.0	11.1	14.6	9.5	8.7	7.0	11.2	0.0	20.3
	Pentacosane	0.0		5.2	4.5	3.5	4.7	3.5	3.2	1.9	2.6	1.5	0.0	11.4	3.9	3.5	2.6	6.3	4.8	3.8	5.5
	11 13 Me C25	5.4		6.1	6.5	7.7	7.2	6.4	9.0	7.3	5.4	6.4	5.9	15.1	6.5	6.4	7.5	9.2	6.7	7.5	6.0
	5 Me C25	3.4		2.0	2.5	2.7	3.1	2.3	2.1	2.9	2.0	3.9	2.2	3.1	2.5	2.2	3.9	3.2	3.4	2.0	2.5
	Heptacosene	3.8		5.1	5.5	3.1	3.8	4.8	7.3	6.3	3.4	5.1	5.0	5.8	4.0	4.1	5.3	4.0	5.0	5.2	5.0

0
0-20
20-40
40-60
60-80
80-100
100+

Figure D.15: Data sheet showing page three of the raw substrate data for the hexadec-9-enoic acid-9,10-d_2 fatty acid experiment.

		Sub 1	Sub 2	Sub 3	Sub 4	Sub 5	Sub 6	Sub 7	Sub 8	Sub 9	Sub 10	Sub 11	Sub 12	Sub 13	Sub 14	Sub 15	Sub 16	Sub 17	Sub 18	Sub 19	Sub 20	Sub 21
Abundance of M	Tricosane	10602	26112	16043	6101	18192	13636	18640	9646	3963	4218	5536		19248	20632	16173	20592	8376	17464	11800		9091
	Pentacosene	45808	256768	204032	90760	168064	157376	193344	118240	26880	47408	61784		285969	335552	249152	276672	157056	146624	131712		109184
	Pentacosene : 2	752	2281	5558	8534	2295	5156	9470	5552	1594	1220	1315		920	3406	1966	3785	981	998	1456		1535
	Pentacosane	5955	8555	5831	1947	4542	5064	5076	4207	2295	1400	3512		6296	8176	6030	8966	3652	7944	3598		3355
	11 13 Me C25	9084	43232	41928	13911	24480	34464	27512	18504	2825	6278	6171		40720	51008	42992	40168	19416	19696	14041		12989
	5 Me C25	36472	183168	148480	13911	108128	125720	124848	89376	16295	38296	50240		199744	249792	182528	199424	116640	116128	94672		70200
	Heptacosene	6784	43256	35424	14319	24728	26976	25176	21040	2810	6616	7810		42512	54704	36744	40664	25448	22648	25704		17768
Abundance of M+1	Tricosane	3048	4991	3618	2029	3896	2274	4660	2343	1933	807	1692		4801	5024	2508	4184	1254	2830	2845		3003
	Pentacosene	11270	67200	54304	24232	42912	44528	48296	27792	7225	14401	16888		74536	89512	61984	66584	40288	44496	33152		34008
	Pentacosene : 2	171	696	1098	2182	338	1781	2671	2455	461	510	335		492	950	418	787	546	237	474		725
	Pentacosane	660	2469	1326	572	1682	1153	1304	1655	865	622	828		1637	2394	1628	2587	1022	2031	1488		951
	11 13 Me C25	4381	24016	22736	9848	15467	14252	15297	7843	1681	3433	2958		22208	28000	22208	18608	8203	8953	7005		5883
	5 Me C25	6563	37888	35472	9848	23120	24448	30652	18016	3909	9488	10461		44376	55608	35200	43408	25184	22960	19128		12811
	Heptacosene	1674	13737	10754	3560	7219	6083	8110	6399	764	2156	2854		11214	15071	12635	11551	6232	6321	4937		5932
Abundance of M+2	Tricosane	553	443	838	287	311	339	308	579		257			909	463	498	727		329	687		284
	Pentacosene	1290	10475	7557	3423	6550	5322	7838	3758	790	1027	2474		8435	11294	8256	9658	4509	5848	4397		2732
	Pentacosene : 2				845			201	359								202					
	Pentacosane	317	211	438		178		377				186		353		236	255		202			
	11 13 Me C25	847	436	2829	964	1490	1797	1662	1075	216	427	925		3035	3399	2768	2339	1472	1346	1083		1088
	5 Me C25	1174	4298	4133	964	2312	2502	3582	1783	945	1261	1089		4202	6054	3559	5295	2144	2398	2573		1763
	Heptacosene	355	2077	1616	648	850	1017	1142	844	175	236	412		1528	802	2122	1938	1602	1687	1114		895
Percentage of M+1	Tricosane	28.7	19.1	22.6	33.3	21.4	16.7	25.0	24.3	48.8	19.1	30.6		24.9	24.4	15.5	20.3	15.0	16.2	24.1		33.0
	Pentacosene	24.6	26.2	26.6	26.7	25.5	28.3	25.0	23.5	26.9	30.4	27.3		26.1	26.7	24.9	24.1	25.7	30.3	25.2		31.1
	Pentacosene : 2	22.7	30.5	19.8	25.6	14.7	34.5	28.2	44.2	28.9	41.8	25.5		53.5	27.9	21.3	20.8	55.7	23.7	32.6		47.2
	Pentacosane	11.1	28.9	22.7	29.4	37.0	22.8	25.7	39.3	37.7	44.4	23.6		26.0	29.3	27.0	28.9	28.0	25.6	41.4		28.3
	11 & 13 Me C25	48.2	55.6	54.2	70.8	63.2	41.4	55.6	42.4	59.5	54.7	47.9		54.5	54.9	51.7	46.3	42.2	45.5	49.9		45.3
	5 Me C25	18.0	20.7	23.9	70.8	21.4	19.4	24.6	20.2	24.0	24.8	20.8		22.2	22.3	19.3	21.8	21.6	19.8	20.2		18.2
	Heptacosene	24.7	31.8	30.4	24.9	29.2	22.5	32.2	30.4	27.2	32.6	36.5		26.4	27.6	34.4	28.4	24.5	27.9	19.2		33.4
Percentage of M+2	Tricosane	5.2	1.7	5.2	4.7	1.7	2.5	1.7	6.0	0.0	6.1	0.0		4.7	2.2	3.1	3.5	0.0	1.9	5.8		3.1
	Pentacosene	2.8	4.1	3.7	3.8	3.9	3.4	4.1	3.2	2.9	2.2	4.0		2.9	3.4	3.3	3.5	2.9	4.0	3.3		2.5
	Pentacosene : 2	0.0	0.0	0.0	9.9	0.0	0.0	2.1	6.5	0.0	0.0	0.0		0.0	0.0	0.0	5.3	0.0	0.0	0.0		0.0
	Pentacosane	5.3	2.5	7.5	0.0	3.9	0.0	7.4	0.0	0.0	0.0	5.3		5.6	0.0	3.9	2.8	0.0	2.5	0.0		0.0
	11 13 Me C25	9.3	1.0	6.7	6.9	6.1	5.2	6.0	5.8	7.6	6.8	15.0		7.5	6.7	6.4	5.8	7.6	6.8	7.7		8.4
	5 Me C25	3.2	2.3	2.8	6.9	2.1	2.0	2.9	2.0	5.8	3.3	2.2		2.1	2.4	1.9	2.7	1.8	2.1	2.7		2.5
	Heptacosene	5.2	4.8	4.6	4.5	3.4	3.8	4.5	4.0	6.2	3.6	5.3		3.6	1.5	5.8	4.8	6.3	7.4	4.3		5.0

0
0-20
20-40
40-60
60-80
80-100
100+

Figure D.16: Data sheet showing page one of the raw substrate data for the hexadec-11-enoic acid-11,12-d_2 fatty acid experiment.

		Sub 22	Sub 23	Sub 24	Sub 25	Sub 26	Sub 27	Sub 28	Sub 29	Sub 30	Sub 31	Sub 32	Sub 33	Sub 34	Sub 35	Sub 36	Sub 37	Sub 38	Sub 39	Sub 40	Sub 41	Sub 42
Abundance of M	Tricosane	18968	11150	18208	9192	8363	10258	11500	4460	19000	14891	12025		16174	17920	3782	22304		58936	16576	15744	21928
	Pentacosene	224064	97408	104192	21344	59096	79000	58160	31728	141312	105792	125384		154176	172480	26400	160768		624192	151680	115008	192832
	Pentacosene : 2	1151	1377	1255	1194	1602	1057	932	731	3248	1096	1454		1010	1320	1202	1266		4502	1417	3203	2759
	Pentacosane	5636	5400	9493	5038	3952	4024	3817	2733	10791	4130	7777		7445	7706	1987	12785		36344	6367	8945	10039
	11 13 Me C25	24576	11409	7267	1735		8101	3893	3935	11892	6694	8225		22968	22784	1898	9936		63456	18760	8236	23200
	5 Me C25	151168	72032	56760	19000	33848	50208	25768	24288	84608	47704	67624		121224	117040	14004	86240		384960	111144	66584	141888
	Heptacosene	34144	14788	16648	2394	6116	9247	5269	4609	25904	10043	16416		23600	26664	4152	24208		101792	24800	14618	30160
Abundance of M+1	Tricosane	5566	4636	3808	2395	2209	2918	2820	1581	5032	3255	3641		4227	5290	1153	5812		15713	4532	4340	5582
	Pentacosene	61624	27832	24680	7150	15370	19408	17448	10337	35960	29368	29392		48816	51976	6119	43672		159488	40696	31008	49176
	Pentacosene : 2	471	296	499	255	191	204	373	156	1111	437	303		451	439	645	344		1151	403	477	981
	Pentacosane	1607	1834	2399	1068	1301	1121	1958	991	2292	1730	1992		2364	3128	761	3212		10407	1842	3086	2621
	11 13 Me C25	14078	7800	4130	1190		3036	915	1874	6821	3366	4294		11502	12409	903	5736		32436	10667	4889	15068
	5 Me C25	33168	15432	15261	4295	5964	11970	7013	5995	16840	12462	13770		26192	25592	2349	19160		86168	23064	16190	31312
	Heptacosene	10108	4003	3489	1272	2234	3159	2545	1888	6640	3760	5871		7740	5874	688	7206		29560	7310	5739	9021
Abundance of M+2	Tricosane	829	444	536	164	513	488	657		531	709	481			872		760		1720	557	654	1250
	Pentacosene	7629	4097	4799	1237	2164	3215	1393	1343	6009	4058	5292		6122	5962	1380	6555		22584	6388	3954	8359
	Pentacosene : 2	209	175																390			
	Pentacosane	349		811	267			169		576	180			355	287	294	452		1671	150	272	525
	11 13 Me C25	2210	477	969			225		274	1373	850	1189		1932	1272	172			5263	1441	651	2030
	5 Me C25	4663	2676	1489	650	1072	1397	764	1259	2296	1752	2447		2342	2129	468	1975		8701	3122	2449	3789
	Heptacosene	1749	157	731		511	434	179	377	717	242	924		1066	1455		734		4516	1076	979	1951
Percentage of M+1	Tricosane	29.3	41.6	20.9	26.1	26.4	28.4	24.5	35.4	26.5	21.9	30.3		26.1	29.5	30.5	26.1		26.7	27.3	27.6	25.5
	Pentacosene	27.5	28.6	23.7	33.5	26.0	24.6	30.0	32.6	25.4	27.8	23.4		31.7	30.1	23.2	27.2		25.6	26.8	27.0	25.5
	Pentacosene : 2	40.9	21.5	39.8	21.4	11.9	19.3	40.0	21.3	34.2	39.9	20.8		44.7	33.3	53.7	27.2		25.6	28.4	14.9	35.6
	Pentacosane	28.5	34.0	25.3	21.2	32.9	27.9	51.3	36.3	21.2	41.9	25.6		31.8	40.6	38.3	25.1		28.6	28.9	34.5	26.1
	11 & 13 Me C25	57.3	68.4	56.8	68.6		37.5	23.5	47.6	57.4	50.3	52.2		50.1	54.5	47.6	57.7		51.1	56.9	59.4	64.9
	5 Me C25	21.9	21.4	26.9	22.6	17.6	23.8	27.2	24.7	19.9	26.1	20.4		21.6	21.9	16.8	22.2		22.4	20.8	24.3	22.1
	Heptacosene	29.6	27.1	21.0	53.1	36.5	34.2	48.3	41.0	25.6	37.4	35.8		32.8	22.0	16.6	29.8		29.0	29.5	39.3	29.9
Percentage of M+2	Tricosane	4.4	4.0	2.9	1.8	6.1	4.8	5.7	0.0	2.8	4.8	4.0		0.0	4.9	0.0	3.4		2.9	3.4	4.2	5.7
	Pentacosene	3.4	4.2	4.6	5.8	3.7	4.1	2.4	4.2	4.3	3.8	4.2		4.0	3.5	5.2	4.1		3.6	4.2	3.4	4.3
	Pentacosene : 2	18.2	12.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0		8.7	0.0	0.0	0.0
	Pentacosane	6.2	0.0	8.5	5.3	0.0	0.0	4.4	0.0	5.3	4.4	0.0		4.8	3.7	14.8	3.5		4.6	2.4	3.0	5.2
	11 13 Me C25	9.0	4.2	13.3	0.0		2.8	0.0	7.0	11.5	12.7	14.5		8.4	5.6	9.1	0.0		8.3	7.7	7.9	8.8
	5 Me C25	3.1	3.7	2.6	3.4	3.2	2.8	3.0	5.2	2.7	3.7	3.6		1.9	1.8	3.3	2.3		2.3	2.8	3.7	2.7
	Heptacosene	5.1	1.1	4.4	0.0	8.4	4.7	3.4	8.2	2.8	2.4	5.6		4.5	5.5	0.0	3.0		4.4	4.3	6.7	6.5

0
0-20
20-40
40-60
60-80
80-100
100+

Figure D.17: Data sheet showing page two of the raw substrate data for the hexadec-11-enoic acid-11,12-d_2 fatty acid experiment.

		Sub 1	Sub 2	Sub 3	Sub 4	Sub 5	Sub 6	Sub 7	Sub 8	Sub 9	Sub 10	Sub 11	Sub 12	Sub 13	Sub 14	Sub 15	Sub 16	Sub 17	Sub 18	Sub 19	Sub 20	Sub 21	Sub 22	Sub 23	Sub 24	Sub 25
Abundance of M	Tricosane	14177	47336	8497	57192	49256	10545	37264	31664	49224	78416	10108	135616	27320	37936	29152	38280	59488	79456	20248	69064	101128	117848	53160	52768	69016
	Pentacosene	124208	357632	113400	303680	422720	121952	246720	214592	488960	334592	156928	557824	242432	292608	348096	286656	390080	543360	203840	588544	659584	940544	386432	662208	413248
	Pentacosene : 2	12495	17488	8119	10326	17424	5311	5874	7561	15959	9348	5338	21184	6119	5926	7696	5902	9399	13192	5898	6289	38864	15127	9808	6205	5774
	Pentacosane	6861	15281	5090	31200	18704	5831	16304	10784	25992	18848	5290	62160	22376	19568	21144	36904	27896	18792	7347	41816	48272	40560	21656	28312	24880
	11 13 Me C25	6206	45136	8174	27696	88528	10864	26808	38288	75984	49600	8603	68352	10894	32328	24408	17880	26480	155072	17680	94648	133952	277568	75032	108808	60632
	5 Me C25	46936	195968	42488	123496	230080	43824	126040	77336	328064	152384	78184	304512	144104	155776	168384	174720	175616	327296	93984	387584	416256	644032	176832	455168	225664
	Heptacosene	2123	36616	1149	34184	68104	5172	17856	34352	93184	42160	9571	93368	24448	33712	43640	41568	50584	112040	24720	109192	126152	176064	76288	125920	66328
Abundance of M+1	Tricosane	4485	9280	1891	16616	14420	4950	7883	8533	13673	16063	5362	34816	8433	9570	7627	12481	15303	17888	9822	19176	29840	29496	12680	14993	18344
	Pentacosene	31464	89888	36912	75824	115000	32888	68744	63528	124792	90016	35400	162432	64376	83008	83608	75232	106464	147072	60040	161856	171456	263936	106712	179200	106984
	Pentacosene : 2	3452	3944	829	3423	3090	524	1592	1402	4111	2171	1023	6054	1658	2766	2394	2282	1973	2932	1728	868	10957	5453	3482	1449	2350
	Pentacosane	3457	3636	1621	6787	5028	1150	2212	2427	4123	6628	1405	14810	7513	4513	4339	8499	7609	7013	3729	8828	12438	17336	5092	9937	9631
	11 13 Me C25	3188	20232	5763	13055	44728	5800	19832	25296	40304	27040	5357	31576	6087	15829	13387	12057	15884	76248	5990	55704	77504	143680	37480	56872	37248
	5 Me C25	14569	42480	9308	20952	46560	11656	33016	18600	72752	37320	18848	55968	24456	40200	38520	31072	35032	80328	17824	83224	85280	142976	38336	97296	53800
	Heptacosene	237	13437	695	7886	18992	1685	9367	9058	25592	11972	2761	32392	7763	7596	12145	12747	14453	34560	8447	34248	43272	50520	23968	37000	17728
Abundance of M+2	Tricosane	757	1262	464	2879	2639	1079	625	331	1280	2135	846	4453	1812	791	660	558	2623	1816	155	5748	2998	6265	1501	1702	3448
	Pentacosene	3079	20896	10939	10745	23400	6596	15697	11761	29248	15132	7220	26344	7786	17840	15803	17696	11996	27576	11532	32552	34576	55960	20920	42336	15050
	Pentacosene : 2	328	1684			392	172	996	181	1829	365	569	1286		432	564	1288	177	578	575	730	2823	726	329	586	761
	Pentacosane	765	712		615	732			286				2026	1351	582	466	864	514	389	820	2155	2516	495	657	562	1677
	11 13 Me C25	599	613		1476	4481	422	1531	3699	5734	4472	781	9264	836	1589	749	1585	2302	11533	2797	7224	13500	14599	4814	7754	3750
	5 Me C25	2261	4072	1170	3216	6491	1509	2725	2264	8949	3368	1325	5995	2737	3576	2733	6031	5468	10212	1671	9848	8077	14365	2682	9693	4364
	Heptacosene		1904		1954	4021	365	1051	2225	5258	2526	1507	3677	770	3121	2069	751	2573	5749	1143	5119	9188	9979	2199	7013	4444
Percentage of M+1	Tricosane	31.6	19.6	22.3	29.1	29.3	46.9	21.2	26.9	27.8	20.5	53.0	25.7	30.9	25.2	26.2	32.6	25.7	22.5	48.5	27.8	29.5	25.0	23.9	28.4	26.6
	Pentacosene	25.3	25.1	32.6	25.0	27.2	27.0	27.9	29.6	25.5	26.9	22.6	29.1	26.6	28.4	24.0	26.2	27.3	27.1	29.5	27.5	26.0	28.1	27.6	27.1	25.9
	Pentacosene : 2	27.6	22.6	10.2	33.1	17.7	9.9	27.1	18.5	25.8	23.2	19.2	28.6	27.1	46.7	31.1	38.7	21.0	22.2	29.3	13.8	28.2	36.0	35.5	23.4	40.7
	Pentacosane	50.4	23.8	31.8	21.8	26.9	19.7	13.6	22.5	15.9	35.2	26.6	23.8	33.6	23.1	20.5	23.0	27.3	37.3	50.8	21.1	25.8	42.7	23.5	35.1	38.7
	11 & 13 Me C25	51.4	44.8	70.5	47.1	50.5	53.4	74.0	66.1	53.0	54.5	62.3	46.2	55.9	49.0	54.8	67.4	60.0	49.2	33.9	58.9	57.9	51.8	50.0	52.3	61.4
	5 Me C25	31.0	21.7	21.9	17.0	20.2	26.6	26.2	24.1	22.2	24.5	24.1	18.4	17.0	25.8	22.9	17.8	19.9	24.5	19.0	21.5	20.5	22.2	21.7	21.4	23.8
	Heptacosene	11.2	36.7	60.5	23.1	27.9	32.6	52.5	26.4	27.5	28.4	28.8	34.7	31.8	22.5	27.8	30.7	28.6	30.8	34.2	31.4	34.3	28.7	31.4	29.4	26.7
Percentage of M+2	Tricosane	5.3	2.7	5.5	5.0	5.4	10.2	1.7	1.0	2.6	2.7	8.4	3.3	6.6	2.1	2.3	1.5	4.4	2.3	0.8	8.3	3.0	5.3	2.8	3.2	5.0
	Pentacosene	2.5	5.8	9.6	3.5	5.5	5.4	6.4	5.5	6.0	4.5	4.6	4.7	3.2	6.1	4.5	6.2	3.1	5.1	5.7	5.5	5.2	5.9	5.4	6.4	3.6
	Pentacosene : 2	2.6	9.6	0.0	0.0	2.2	3.2	17.0	2.4	11.5	3.9	10.7	6.1	0.0	7.3	7.3	21.8	1.9	4.4	9.7	11.6	7.3	4.8	3.4	9.4	13.2
	Pentacosane	11.1	4.7	0.0	2.0	3.9	0.0	0.0	2.7	0.0	0.0	0.0	3.3	6.0	3.0	2.2	2.3	1.8	2.1	11.2	5.2	5.2	1.2	3.0	2.0	6.7
	11 13 Me C25	9.7	1.4	0.0	5.3	5.1	3.9	5.7	9.7	7.5	9.0	9.1	13.6	7.7	4.9	3.1	8.9	8.7	7.4	15.8	7.6	10.1	5.3	6.4	7.1	6.2
	5 Me C25	4.8	2.1	2.8	2.6	2.8	3.4	2.2	2.9	2.7	2.2	1.7	2.0	1.9	2.3	1.6	3.5	3.1	3.1	1.8	2.5	1.9	2.2	1.5	2.1	1.9
	Heptacosene	0.0	5.2	0.0	5.7	5.9	7.1	5.9	6.5	5.6	6.0	15.7	3.9	3.1	9.3	4.7	1.8	5.1	5.1	4.6	4.7	7.3	5.7	2.9	5.6	6.7

0
0-20
20-40
40-60
60-80
80-100
100+

Figure D.18: Data sheet showing page one of the raw substrate data for the octadec-7-enoic acid-7,8-d_2 fatty acid experiment.

		Sub 26	Sub 27	Sub 28	Sub 29	Sub 30	Sub 31	Sub 32	Sub 33	Sub 34	Sub 35	Sub 36	Sub 37	Sub 38	Sub 39	Sub 40	Sub 41	Sub 42	Sub 43	Sub 44	Sub 45	Sub 46	Sub 47	Sub 48	Sub 49	Sub 50
Abundance of M	Tricosane	51888	94168	77728	83512	51520	52144	46936	69752	39576	160768	21896	59328	83928	105920	123920	213504	105416		45264	75616	41376	45656	52464		67888
	Pentacosene	473280	576320	532736	494208	302592	422464	591296	528512	317632	1068032	237248	374272	688000	568560	1190400	1323008	973504		390208	393728	429568	564288	281152		536064
	Pentacosene : 2	10848	14301	5335	6854	4399	11458	8092	3345	4880	17328	3382	6041	5130	12142	8634	14059	16371		3258	7305	4097	8949	4669		20040
	Pentacosane	40064	35920	27528	42120	15665	21856	34912	21368	18472	68024	9701	29728	50792	59072	103560	98832	64320		16464	40584	25128	22392	32024		31856
	11 13 Me C25	76080	177024	161664	72416	87192	30424	50168	141248	31752	324544	41880	50144	108536	83608	205120	231872	322560		126704	29976	24248	80008	25840		76704
	5 Me C25	216960	372160	340288	266688	190848	237056	339648	307456	202176	685696	121424	228416	472704	344000	766848	761600	526400		219072	154624	223168	404352	204544		307264
	Heptacosene	81816	120864	107304	79752	42608	65288	105144	108120	53816	251264	53792	56072	155072	126664	264576	294848	266624		90704	61584	54176	105744	44256		109360
Abundance of M+1	Tricosane	16380	22200	18320	17312	12010	10609	12661	15845	9788	38848	6583	16408	27680	26464	24712	41160	28064		10150	18992	8614	12306	12874		20512
	Pentacosene	133376	154368	146176	135936	87288	112792	148096	138496	95680	280576	63808	99536	191872	170296	297664	357568	277312		101448	103312	106168	148608	87528		149248
	Pentacosene : 2	3312	3961	424	238	2032	3370	2427	1983	857	6350	884	1904	2677	2011	2379	3631	4648		759	1392	822	3908	1789		3215
	Pentacosane	8232	10930	11031	10316	4176	7007	11993	5245	5502	25512	2922	7045	16142	15822	27960	27360	16448		4453	11148	6746	7692	6834		7369
	11 13 Me C25	44368	85112	88512	35888	46304	15688	34232	82152	14294	187776	27520	24688	47432	40640	118208	125264	178752		71488	17832	11149	46424	17808		39464
	5 Me C25	41120	72824	76720	55704	38648	47944	76808	74192	48600	131200	30192	42872	102416	80080	163200	183040	112464		48192	37328	49184	97144	47424		64504
	Heptacosene	21720	34712	31312	24064	12369	17864	33760	28880	16172	79144	14707	16680	44344	39256	75920	95224	87528		25056	20592	21152	29392	8261		34000
Abundance of M+2	Tricosane	751	2599	2002	3528	2054	1925	2733	2495	920	3774	1249	1434	3646	2217	4485	5681	3309		597	2227	1277	1406	1536		1934
	Pentacosene	20248	30168	27064	25016	12583	22544	28336	28384	16257	50920	11279	17472	47296	29536	53888	53480	41208		16584	16133	18672	42792	33960		23648
	Pentacosene : 2	515	293	603		255	871	165	229		263				937	479	641	420			428	408	1805	457		
	Pentacosane	2054	2873	1679	1756	1311	1856	2344	641	1532	2895	174	2150	2673	2719	3439	2903	1053		875	3002	201	1294	773		1363
	11 13 Me C25	4846	10398	8821	5518	6418	3126	3339	9563	3580	27904	2921	3624	5431	5399	12966	15193	24792		8702	3024	1577	6476	1595		5204
	5 Me C25	5851	9737	7935	7656	5419	5888	8786	7439	4496	14102	3118	1920	10430	8585	18024	20424	10734		3675	3666	49184	11583	9049		8710
	Heptacosene	1916	6992	5880	5358	2363	2607	6553	3102	1668	13609	2581	5108	5563	7416	17216	15291	9960		4222	1775	2802	6710	3471		3777
Percentage of M+1	Tricosane	31.6	23.6	23.6	20.7	23.3	20.3	27.0	22.7	24.7	24.2	30.1	27.7	33.0	25.0	19.9	19.3	26.6		22.4	25.1	20.8	27.0	24.5		30.2
	Pentacosene	28.2	26.8	27.4	27.5	28.8	26.7	25.0	26.2	30.1	26.3	26.9	26.6	27.9	30.0	25.0	27.0	28.5		26.0	26.2	24.7	26.3	31.1		27.8
	Pentacosene : 2	30.5	27.7	7.9	3.5	46.2	29.4	30.0	59.3	17.6	36.6	26.1	31.5	52.2	16.6	27.6	25.8	28.4		23.3	19.1	20.1	43.7	38.3		16.0
	Pentacosane	20.5	30.4	40.1	24.5	26.7	32.1	34.4	24.5	29.8	37.5	30.1	23.7	31.8	26.8	27.0	27.7	25.6		27.0	27.5	26.8	34.4	21.3		23.1
	11 & 13 Me C25	58.3	48.1	54.8	49.6	53.1	51.6	68.2	58.2	45.0	57.9	65.7	49.2	43.7	48.6	57.6	54.0	55.4		56.4	59.5	46.0	58.0	68.9		51.4
	5 Me C25	19.0	19.6	22.5	20.9	20.3	20.2	22.6	24.1	24.0	19.1	24.9	18.8	21.7	23.3	21.3	24.0	21.4		22.0	24.1	22.0	24.0	23.2		21.0
	Heptacosene	26.5	28.7	29.2	30.2	29.0	27.4	32.1	26.7	30.1	31.5	27.3	29.7	28.6	31.0	28.7	32.3	32.8		27.6	33.4	39.0	27.8	18.7		31.1
Percentage of M+2	Tricosane	1.4	2.8	2.6	4.2	4.0	3.7	5.8	3.6	2.3	2.3	5.7	2.4	4.3	2.1	3.6	2.7	3.1		1.3	2.9	3.1	3.1	2.9		2.8
	Pentacosene	4.3	5.2	5.1	5.1	4.2	5.3	4.8	5.4	5.1	4.8	4.8	4.7	6.9	5.2	4.5	4.0	4.2		4.3	4.1	4.3	7.6	12.1		4.4
	Pentacosene : 2	4.7	2.0	11.3	0.0	5.8	7.6	2.0	6.8	0.0	1.5	0.0	0.0	0.0	7.7	5.5	4.6	2.6		0.0	5.9	10.0	20.2	9.8		0.0
	Pentacosane	5.1	8.0	6.1	4.2	8.4	8.5	6.7	3.0	8.3	4.3	1.8	7.2	5.3	4.6	3.3	2.9	1.6		5.3	7.4	0.8	5.8	2.4		4.3
	11 13 Me C25	6.4	5.9	5.5	7.6	7.4	10.3	6.7	6.8	11.3	8.6	7.0	7.2	5.0	6.5	6.3	6.6	7.7		6.9	10.1	6.5	8.1	6.2		6.8
	5 Me C25	2.7	2.6	2.3	2.9	2.8	2.5	2.6	2.4	2.2	2.1	2.6	0.8	2.2	2.5	2.4	2.7	2.0		1.7	2.4	22.0	2.9	4.4		2.8
	Heptacosene	2.3	5.8	5.5	6.7	5.5	4.0	6.2	2.9	3.1	5.4	4.8	9.1	3.6	5.9	6.5	5.2	3.7		4.7	2.9	5.2	6.3	7.8		3.5

0
0-20
20-40
40-60
60-80
80-100
100+

Figure D.19: Data sheet showing page two of the raw substrate data for the octadec-7-enoic acid-7,8-d_2 fatty acid experiment.

		Sub 51	Sub 52	Sub 53	Sub 54	Sub 55	Sub 56	Sub 57	Sub 58	Sub 59	Sub 60	Sub 61	Sub 62	Sub 63	Sub 64	Sub 65	Sub 66	Sub 67	Sub 68	Sub 69	Sub 70	Sub 71	Sub 72	Sub 73	Sub 74	Sub 75	Sub 76	Sub 77
Abundance of M	Tricosane	125016	97072	111248	132800	60304	140992	113912	91112	86032		56136	132160	48544	144576	47848	89888	78248	80136	68640	188992	72064	78312	51024	54640	53696	61056	28416
	Pentacosene	1087488	721728	799616	978112	548864	771648	734016	760000	647936		406144	885248	476160	1030336	416640	748160	507072	604416	500224	1121280	532096	678592	452160	420288	382080	502144	209408
	Pentacosene : 2	23720	12281	29752	55520	42768	42648	117384	117304	46080		8533	86816	13187	76480	11553	32520	40856	69208	59368	120096	61712	97152	32120	33824	37088	76888	11982
	Pentacosane	45624	39552	66080	55056	29744	59608	64592	48704	41944		27624	55432	20040	57976	27288	47312	32056	42952	26576	83400	21360	49384	17560	26712	29864	24056	12022
	11 13 Me C25	145152	97544	133376	334848	211968	135488	166528	126944	125488		42832	180864	76888	274432	37688	211712	139520	71664	136064	248896	82584	100048	91976	69704	36768	137216	25408
	5 Me C25	635456	470016	548096	585472	314240	402304	446400	522176	488832		273536	518464	338496	560512	263680	447744	307840	343232	245312	788480	370112	391872	239488	203008	236928	291392	82056
	Heptacosene	224384	144768	198656	251072	156032	192960	151872	146048	145216		74664	190848	67768	254912	85664	190720	113328	112008	111088	241088	96512	132416	89440	109432	68912	100448	24304
Abundance of M+1	Tricosane	29392	20648	24784	37912	14038	33400	28120	23864	22712		13649	29808	11335	30784	12379	24624	23264	22536	17400	47904	19904	23328	13395	15872	14135	19040	7881
	Pentacosene	284096	182272	211264	252544	142720	208960	206272	199104	164096		117688	240960	122032	253760	119416	201216	141760	160640	128024	287744	138752	192640	126808	120280	105304	129552	57192
	Pentacosene : 2	4997	2311	10138	13174	5765	9585	29408	23904	15061		3032	28920	2679	20208	1495	9274	11365	21056	15339	27328	16314	28456	9863	11543	10930	15667	4446
	Pentacosane	14993	7250	18720	15119	10503	21872	16728	14178	11057		8213	15955	3400	14648	9618	11657	8362	12885	10004	27256	8226	12303	6301	5968	6404	6020	2594
	11 13 Me C25	88848	53560	72520	186688	110264	75544	91824	74712	70712		22656	102216	38688	148032	21528	120376	76944	39752	74568	137728	46344	50240	50016	38616	26336	71624	12835
	5 Me C25	150400	95920	114544	134912	71568	88408	92888	117208	118712		65984	107456	83744	116976	58240	99328	70400	67814	51520	155328	76720	92752	53192	36048	55816	62576	17128
	Heptacosene	55720	47656	44232	68944	43144	47032	53936	43688	37904		24000	59176	25656	71936	25264	51744	27864	27752	33152	73720	31232	43520	23536	31224	22240	27496	7947
Abundance of M+2	Tricosane	4151	2634	2779	4671	1762	5896	3794	1937	2092		2725	4848	2660	7387	2372	2843	2312	1451	1043	4824	1457	1988	1704	1827	2558	355	1596
	Pentacosene	50144	40952	44816	41600	26968	33224	37608	35208	46680		19768	53208	28296	38640	17912	42840	30384	27960	22544	48440	39008	33816	17728	13203	21216	24088	7235
	Pentacosene : 2	836	813	2033	2892	2351	1539	5028	6754	2964		1094	4255	1278	5283	1093	1188	1889	1430	3396	3400	1953	6751	1199	771	1901	2174	466
	Pentacosane	1411	1427	2989	2845	1781	3257	3465	1559	1706		414	3302	371	3712	995	1452	1499	1057	363	3526	1216	2846	775	723	1536	2096	499
	11 13 Me C25	13029	7360	7952	20728	13387	8031	11636	8069	7671		3647	12520	6133	20704	5841	16624	8752	5745	8694	18240	7586	5364	7012	3031	4323	9417	1775
	5 Me C25	15459	9244	20088	14404	7235	8865	12023	11907	11129		6526	13902	6510	12200	8857	9558	10793	5849	6453	18280	10699	9298	5752	4687	7462	5461	2509
	Heptacosene	9473	7745	11418	12681	6164	7242	7594	7505	7411		5161	12128	5790	11737	4821	8346	6238	5319	3001	13581	5317	6688	4990	4316	7471	5255	669
Percentage of M+1	Tricosane	23.5	21.3	22.3	28.5	23.3	23.7	24.7	26.2	26.4		24.3	22.6	23.3	21.3	25.9	27.4	29.7	28.1	25.3	25.3	27.6	29.8	26.3	29.0	26.3	31.2	27.7
	Pentacosene	26.1	25.3	26.4	25.8	26.0	27.1	28.1	26.2	25.3		29.0	27.2	25.6	24.6	28.7	26.9	28.0	26.6	25.6	25.7	26.1	28.4	28.0	28.6	27.6	25.8	27.3
	Pentacosene : 2	21.1	18.8	34.1	23.7	13.5	22.5	25.1	20.4	32.7		35.5	33.3	20.3	26.4	12.9	28.5	27.8	30.4	25.8	22.8	26.4	29.3	30.7	34.1	29.5	20.4	37.1
	Pentacosane	32.9	18.3	28.3	27.5	35.3	36.7	25.9	29.1	26.4		29.7	28.8	17.0	25.3	35.2	24.6	26.1	30.0	37.6	32.7	38.5	24.9	35.9	22.3	21.4	25.0	21.6
	11 & 13 Me C25	61.2	54.9	54.4	55.8	52.0	55.8	55.1	58.9	56.3		52.9	56.5	50.3	53.9	57.1	56.9	55.1	55.5	54.8	55.3	56.1	50.2	54.4	55.4	71.6	52.2	50.5
	5 Me C25	23.7	20.4	20.9	23.0	22.8	22.0	20.8	22.4	24.3		24.1	20.7	24.7	20.9	22.1	22.2	22.9	19.8	21.0	19.7	20.7	23.7	22.2	17.8	23.6	21.5	20.9
	Heptacosene	24.8	32.9	22.3	27.5	27.7	24.4	35.5	29.9	26.1		32.1	31.0	37.9	28.2	29.5	27.1	24.6	24.8	29.8	30.6	32.4	32.9	26.3	28.5	32.3	27.4	32.7
Percentage of M+2	Tricosane	3.3	2.7	2.5	3.5	2.9	4.2	3.3	2.1	2.4		4.9	3.7	5.5	5.1	5.0	3.2	3.0	1.8	1.5	2.6	2.0	2.5	3.3	3.3	4.8	0.6	5.6
	Pentacosene	4.6	5.7	5.6	4.3	4.9	4.3	5.1	4.6	7.2		4.9	6.0	5.9	3.8	4.3	5.7	6.0	4.6	4.5	4.3	7.3	5.0	3.9	3.1	5.6	4.8	3.5
	Pentacosene : 2	3.5	6.6	6.8	5.2	5.5	3.6	4.3	5.8	6.4		12.8	4.9	9.7	6.9	9.5	3.7	4.6	2.1	5.7	2.8	3.2	6.9	3.7	2.3	5.1	2.8	3.9
	Pentacosane	3.1	3.6	4.5	5.2	6.0	5.5	5.4	3.2	4.1		1.5	6.0	1.9	6.4	3.6	3.1	4.7	2.5	1.4	4.2	5.7	5.8	4.4	2.7	5.1	8.7	4.2
	11 13 Me C25	9.0	7.5	6.0	6.2	6.3	5.9	7.0	6.4	6.1		8.5	6.9	8.0	7.5	15.5	7.9	6.3	8.0	6.4	7.3	9.2	5.4	7.6	4.3	11.8	6.9	7.0
	5 Me C25	2.4	2.0	3.7	2.5	2.3	2.2	2.7	2.3	2.3		2.4	2.7	1.9	2.2	3.4	2.1	3.5	1.7	2.6	2.3	2.9	2.4	2.4	2.3	3.1	1.9	3.1
	Heptacosene	4.2	5.3	5.7	5.1	4.0	3.8	5.0	5.1	5.1		6.9	6.4	8.5	4.6	5.6	4.4	5.5	4.7	2.7	5.6	5.5	5.1	5.6	3.9	10.8	5.2	2.8

0
0-20
20-40
40-60
60-80
80-100
100+

Figure D.20: Data sheet showing page three of the raw substrate data for the octadec-7-enoic acid-7,8-d_2 fatty acid experiment.

		Sub 1	Sub 2	Sub 3	Sub 4	Sub 5	Sub 6	Sub 7	Sub 8	Sub 9	Sub 10	Sub 11	Sub 12	Sub 13	Sub 14	Sub 15	Sub 16	Sub 17	Sub 18	Sub 19	Sub 20	Sub 21	Sub 22	Sub 23	Sub 24	Sub 25
Abundance of M	Tricosane	17416	22920	9751	15224	32984	23272	31984	35712	13261	19256	4619	13231	8705		8887	9668	30568	12872	15454	6921	15643	20056	9132	20664	59008
	Pentacosene	81392	161280	41256	43640	187328	131904	186048	209984	65752	87976	26960	101648	74192		32928	76592	118104	82248	112800	40672	91792	109192	47704	94936	241088
	Pentacosene : 2	2640	1588	1142	751	4139	1689	1861	5190	1575	1403	732	2943	1357		864	684	2716	2064	1304	715	1225	2367	840	1445	3870
	Pentacosane	9137	19504	3738	19008	9960	8533	12637	22288	9511	7876	2285	6681	8357		6382	7054	22400	8002	8458	3523	9462	12023	8766	12759	30104
	11 13 Me C25	26816	76472	6527	10215	76744	41896	79336	81560	18616	49664	9995	50952	4152		8109	32088	48176	32360	68000	5978	29368	44624	14043	33064	65448
	5 Me C25	75720	143680	32400	42712	149888	73872	130736	167296	64320	63632	27904	85768	60168		30064	43368	153408	49232	90440	28776	78920	90896	44184	118024	242752
	Heptacosene	8011	37216	3611	6767	37280	23080	50648	48776	10233	24624	2660	24064	18576		4726	18624	24544	17168	35032	4909	23912	22248	5516	19568	50544
Abundance of M+1	Tricosane	3645	10424	1755	3026	8137	5358	7885	10676	2721	5092	1811	3498	2695		1121	2500	7257	3966	3310	1113	4284	4216	3422	5959	13990
	Pentacosene	21952	43200	13775	14160	50496	30504	49832	55288	19184	24392	11409	21232	14746		8678	20168	38976	24168	28856	9772	21600	28400	12231	26728	69536
	Pentacosene : 2	1570	1404	166	720	1168	941	2647	2111	582	418	873	488	735		182	943	1132	990	744	270	514	251	360	927	1020
	Pentacosane	2452	2902	1566	2750	3210	3909	2409	6167	1298	3255	631	1975	1164		2601	1952	6114	2811	1668	1573	2255	2900	1421	3866	9980
	11 13 Me C25	17536	45816	3131	5814	32288	21392	42408	39280	9166	21912	5898	26584	1989		7140	21016	29816	18280	38232	5913	16097	25760	7819	17440	46552
	5 Me C25	12483	30184	7382	9959	36928	15411	31800	40096	12828	14234	7788	16384	11360		9413	12257	33832	12160	20248	5051	17864	19024	10632	24704	57736
	Heptacosene	2702	10783	2672	2429	8813	9885	16576	17328	2179	7683	1126	6729	6769		3335	5338	6210	6066	12578	1398	5587	6921	1393	8093	15655
Abundance of M+2	Tricosane	746	1284		819		273	1881	1114	794	210		155	822		387	221	1265	574	274	309	582	833	688	438	1541
	Pentacosene	2584	5145	878	1336	5269	7110	6169	11450	3012	2147	1804	3166	3143		1485	2436	3728	2854	4319	874	3615	3630	968	2800	7288
	Pentacosene : 2	329	4821	1334	1037	7855	3378	9973	3670	1879	1956	801	1784	2251		1505	756	4499	2629	3574	452	2487	4248	1424	3304	8036
	Pentacosane	290	814			428		1091	403	275	286					155		1476		826	253		491		774	1268
	11 13 Me C25	1394	5522			4674	2570	6382	6436	1891	3550	675	5585	691		2158	2143	4644	2353	3562	552	2448	4001	1346	1800	6540
	5 Me C25	2583	3402	1313	2302	3191	1853	5952	4471	1952	2771	747	2694	683		1735	773	7268	1486	2397	404	1718	3149	1242	2243	6755
	Heptacosene		1628			2209	2001	2907	1758	484	911	348	846	882			307	1253	836	1315	333	1374	2076	419	1258	2468
Percentage of M+1	Tricosane	20.9	45.5	18.0	19.9	24.7	23.0	24.7	29.9	20.5	26.4	39.2	26.4	31.0		12.6	25.9	23.7	30.8	21.4	16.1	27.4	21.0	37.5	28.8	23.7
	Pentacosene	27.0	26.8	33.4	32.4	27.0	23.1	26.8	26.3	29.2	27.7	42.3	20.9	19.9		26.4	26.3	33.0	29.4	25.6	24.0	23.5	26.0	25.6	28.2	28.8
	Pentacosene : 2	59.5	88.4	14.5	95.9	28.2	55.7	142.2	40.7	37.0	29.8	119.3	16.6	54.2		21.1	137.9	41.7	48.0	57.1	37.8	42.0	10.6	42.9	64.2	26.4
	Pentacosane	26.8	14.9	41.9	14.5	32.2	45.8	19.1	27.7	13.6	41.3	27.6	29.6	13.9		40.8	27.7	27.3	35.1	19.7	44.6	23.8	24.1	16.2	30.3	33.2
	11 & 13 Me C25	65.4	59.9	48.0	56.9	42.1	51.1	53.5	48.2	49.2	44.1	59.0	52.2	47.9		88.1	65.5	61.9	56.5	56.2	98.9	54.8	57.7	55.7	52.7	71.1
	5 Me C25	16.5	21.0	22.8	23.3	24.6	20.9	24.3	24.0	19.9	22.4	27.9	19.1	18.9		31.3	28.3	22.1	24.7	22.4	17.6	22.6	20.9	24.1	20.9	23.8
	Heptacosene	33.7	29.0	74.0	35.9	23.6	42.8	32.7	35.5	21.3	31.2	42.3	28.0	36.4		70.6	28.7	25.3	35.3	35.9	28.5	23.4	31.1	25.3	41.4	31.0
Percentage of M+2	Tricosane	4.3	5.6	0.0	5.4	0.0	1.2	5.9	3.1	6.0	1.1	0.0	1.2	9.4		4.4	2.3	4.1	4.5	1.8	4.5	3.7	4.2	7.5	2.1	2.6
	Pentacosene	3.2	3.2	2.1	3.1	2.8	5.4	3.3	5.5	4.6	2.4	6.7	3.1	4.2		4.5	3.2	3.2	3.5	3.8	2.1	3.9	3.3	2.0	2.9	3.0
	Pentacosene : 2	12.5	303.6	116.8	138.1	189.8	200.0	535.9	70.7	119.3	139.4	109.4	60.6	165.9		174.2	110.5	165.6	127.4	274.1	63.2	203.0	179.5	169.5	228.7	207.6
	Pentacosane	3.2	4.2	0.0	0.0	4.3	0.0	8.6	1.8	2.9	3.6	0.0	0.0	0.0		2.4	0.0	6.6	0.0	9.8	7.2	0.0	4.1	0.0	6.1	4.2
	11 13 Me C25	5.2	7.2	0.0	0.0	6.1	6.1	8.0	7.9	10.2	7.1	6.8	11.0	16.6		26.6	6.7	9.6	7.3	5.2	9.2	8.3	9.0	9.6	5.4	10.0
	5 Me C25	3.4	2.4	4.1	5.4	2.1	2.5	4.6	2.7	3.0	4.4	2.7	3.1	1.1		5.8	1.8	4.7	3.0	2.7	1.4	2.2	3.5	2.8	1.9	2.8
	Heptacosene	0.0	4.4	0.0	0.0	5.9	8.7	5.7	3.6	4.7	3.7	13.1	3.5	4.7		0.0	1.6	5.1	4.9	3.8	6.8	5.7	9.3	7.6	6.4	4.9

0
0-20
20-40
40-60
60-80
80-100
100+

Figure D.21: Data sheet showing page one of the raw substrate data for the octadec-11-enoic acid-11,12-d_2 fatty acid experiment.

		Sub 26	Sub 27	Sub 28	Sub 29	Sub 30	Sub 31	Sub 32	Sub 33	Sub 34	Sub 35	Sub 36	Sub 37	Sub 38	Sub 39	Sub 40	Sub 41	Sub 42	Sub 43	Sub 44	Sub 45	Sub 46	Sub 47	Sub 48	Sub 49	Sub 50
Abundance of M	Tricosane	23296	35872	19160	37064	116992	8611	9535	17552	22280	21992	22472	77616	28344	19352	14155	38912	20440	36960		24152	35184	51384	81912	31168	15122
	Pentacosene	136448	287680	75928	199936	575936	97136	65528	69408	38304	148352	129304	307200	115640	109200	65696	144192	70256	103256		93904	136000	243392	451968	172096	88736
	Pentacosene : 2	687	4057	2774	3232	20248	1569	321	1971	504	2412	2342	7046	1463	2497	952	1859	873	2026		1350	2648	2625	27712	4442	441
	Pentacosane	15733	39536	18040	17408	69216	7488	6724	11721	25648	12850	9976	31720	9103	9033	15087	18248	11747	26856		13487	13961	33024	46216	19632	14207
	11 13 Me C25	14887	61856	17864	65168	275648	42384	9855	16107	7627	64416	48520	153728	43224	49536	9940	41384	15159	24872		39480	51888	51704	227584	110536	28896
	5 Me C25	125480	291008	79112	181056	573248	65608	78200	81792	42456	125784	126304	269056	124656	99008	74096	156864	75720	128256		113880	183360	256448	522880	199808	102672
	Heptacosene	16167	58424	11383	43616	171776	20024	10597	11657	8321	37768	32944	90752	23720	25176	11461	28648	12096	17968		23872	27288	38448	129872	54056	16496
Abundance of M+1	Tricosane	4242	11038	6931	11567	26752	3994	3642	4158	6771	6489	6240	20000	7167	4457	6188	8020	6270	7542		5762	9164	14149	20048	7963	5397
	Pentacosene	29536	64392	22696	50312	161024	23968	18600	21160	11378	39032	34816	81496	31960	30656	17088	39032	18328	29560		26664	36864	29680	113736	53880	27288
	Pentacosene : 2	565	1241	855	1944	7279	1113	219		494	923	790		893	966		1117	1061			785	1983	2428	12023	1731	877
	Pentacosane	6409	12239	2804	4443	17888	2133	1273	4715	6688	3851	3077	9317	2861	3446	5955	6606	3749	11174		3636	3739	6403	12112	5153	4611
	11 13 Me C25	7611	30896	7214	34264	146880	25792	7383	6594	5406	31896	26888	86096	25832	25352	6371	24936	8978	13428		22512	26352	31680	123504	53056	11875
	5 Me C25	31808	62600	18496	44680	130808	14215	16856	18888	7858	24064	28400	54672	27696	21568	17896	39616	16274	28696		22560	42456	61064	112512	40088	22552
	Heptacosene	4083	17296	4112	11819	57304	7247	2121	2892	1555	15448	11683	27960	6899	8432	3856	10840	1982	5418		7173	7952	11516	36936	20352	4695
Abundance of M+2	Tricosane		1472	310	373	4055			157	388	615	1033	1726	1877	200	1065	783	1128	2153		563	2023	1126	2602	776	430
	Pentacosene	5038	8790	4716	4753	16896	3575	2549	2279	2314	5648	5723	11154	3216	3055	1856	4908	2205	4251		3124	3800	6886	15958	5038	2109
	Pentacosene : 2	2636	11847	4597	6716	45480	6325	2862	3692	1808	6252	6441	22904	6002	3214	5003	5615	4282	3878		5436	6160	12147	55008	23784	4742
	Pentacosane		1867	1082	967	2723	833	675	762	1802	303	431	1206	650	583	603	864	1241	1561		970	862	747	1938	320	480
	11 13 Me C25	995	3894	572	6785	20760	5167	1293	1724	1105	4933	3627	10716	2571	3975	871	3634	2913	2837		1663	3269	5515	19368	7513	2416
	5 Me C25	3177	7659	1969	6772	12542	1573	1071	2221	1912	3644	2678	7601	3734	3394	1820	2520	3823	3960		3479	4779	5275	12472	5014	3201
	Heptacosene		2439	336	1108	4846	988	298	403		1801	628	5029	683	1082	400	1173	546	679		1557	1753	2051	5370	2260	712
Percentage of M+1	Tricosane	18.2	30.8	36.2	31.2	22.9	46.4	38.2	23.7	30.4	29.5	27.8	25.8	25.3	23.0	43.7	20.6	30.7	20.4		23.9	26.0	27.5	24.5	25.5	35.7
	Pentacosene	21.6	22.4	29.9	25.2	28.0	24.7	28.4	30.5	29.7	26.3	26.9	26.5	27.6	28.1	26.0	27.1	26.1	28.6		28.4	27.1	12.2	25.2	31.3	30.8
	Pentacosene : 2	82.2	30.6	30.8	60.1	35.9	70.9	68.2	0.0	98.0	38.3	33.7	0.0	61.0	38.7	0.0	60.1	121.5	0.0		58.1	74.9	92.5	43.4	39.0	198.9
	Pentacosane	40.7	31.0	15.5	25.5	25.8	28.5	18.9	40.2	26.1	30.0	30.8	29.4	31.4	38.1	39.5	36.2	31.9	41.6		27.0	26.8	19.4	26.2	26.2	32.5
	11 & 13 Me C25	51.1	49.9	40.4	52.6	53.3	60.9	74.9	40.9	70.9	49.5	55.4	56.0	59.8	51.2	64.1	60.3	59.2	54.0		57.0	50.8	61.3	54.3	48.0	41.1
	5 Me C25	25.3	21.5	23.4	24.7	22.8	21.7	21.6	23.1	18.5	19.1	22.5	20.3	22.2	21.8	24.2	25.3	21.5	22.4		19.8	23.2	23.8	21.5	20.1	22.0
	Heptacosene	25.3	29.6	36.1	27.1	33.4	36.2	20.0	24.8	18.7	40.9	35.5	30.8	29.1	33.5	33.6	37.8	16.4	30.2		30.0	29.1	30.0	28.4	37.6	28.5
Percentage of M+2	Tricosane	0.0	4.1	1.6	1.0	3.5	0.0	0.0	0.9	1.7	2.8	4.6	2.2	6.6	1.0	7.5	2.0	5.5	5.8		2.3	5.7	2.2	3.2	2.5	2.8
	Pentacosene	3.7	3.1	6.2	2.4	2.9	3.7	3.9	3.3	6.0	3.8	4.4	3.6	2.8	2.8	2.8	3.4	3.1	4.1		3.3	2.8	2.8	3.5	2.9	2.4
	Pentacosene : 2	383.7	292.0	165.7	207.8	224.6	403.1	891.6	187.3	358.7	259.2	275.0	325.1	410.3	128.7	525.5	302.0	490.5	191.4		402.7	232.6	462.7	198.5	535.4	1075.3
	Pentacosane	0.0	4.7	6.0	5.6	3.9	11.1	10.0	6.5	7.0	2.4	4.3	3.8	7.1	6.5	4.0	4.7	10.6	5.8		7.2	6.2	2.3	4.2	1.6	3.4
	11 13 Me C25	6.7	6.3	3.2	10.4	7.5	12.2	13.1	10.7	14.5	7.7	7.5	7.0	5.9	8.0	8.8	8.8	19.2	11.4		4.2	6.3	10.7	8.5	6.8	8.4
	5 Me C25	2.5	2.6	2.5	3.7	2.2	2.4	1.4	2.7	4.5	2.9	2.1	2.8	3.0	3.4	2.5	1.6	5.0	3.1		3.1	2.6	2.1	2.4	2.5	3.1
	Heptacosene	0.0	4.2	3.0	2.5	2.8	4.9	2.8	3.5	0.0	4.8	1.9	5.5	2.9	4.3	3.5	4.1	4.5	3.8		6.5	6.4	5.3	4.1	4.2	4.3

0
0-20
20-40
40-60
60-80
80-100
100+

Figure D.22: Data sheet showing page two of the raw substrate data for the octadec-11-enoic acid-11,12-d_2 fatty acid experiment.

		Sub 50	Sub 51	Sub 52	Sub 53	Sub 54	Sub 55	Sub 56	Sub 57	Sub 58	Sub 59	Sub 60	Sub 61	Sub 62	Sub 63	Sub 64	Sub 65	Sub 66	Sub 67	Sub 68	Sub 69	Sub 70	Sub 71	Sub 72	Sub 73	Sub 74
Abundance of M	Tricosane	15122	8819		4609	7180	15533	15662	5073	63528	34080	46752	16448	14855	17624	12884	16696		17352	21280	16432	15864	9360	6284	26960	15854
	Pentacosene	88736	60632		27728	25896	77184	59488	23112	261952	265984	276736	68312	41968	101696	34168	60192		54720	126656	79168	35696	41464	16371	38088	111520
	Pentacosene : 2	441	1635		701	1029	1421	1023	316	2755	5549	2911	1124	876	1814	1467	1071		804	2859	1300	342	1001	317	818	1759
	Pentacosane	14207	6223		1465	4602	8183	13583	3327	39600	22840	17560	11628	12067	10988	7750	16028		12512	12443	10155	8318	8289	5370	17728	13024
	11 13 Me C25	28896	23056		7027	9769	40712	19712	7189	83480	154816	116776	19312	12307	50568	12221	14413		24640	63280	11374	9416	10001	1881	10847	60464
	5 Me C25	102672	71536		25472	21832	71368	65136	26960	313664	248576	254016	84408	61448	104616	45704	81072		68240	93800	70128	42280	45824	15684	68288	79416
	Heptacosene	16496	9183		3957	3612	18776	9396	2902	52280	78808	62168	11408	6072	22360	5309	11916		11455	34664	10456	4236	5700	1960	4811	30152
Abundance of M+1	Tricosane	5397	2187		394	2144	3429	5866	1622	15479	9538	10921	3038	5782	4027	3884	3408		4441	3170	5495	3198	1723	1053	6133	4942
	Pentacosene	27288	16175		7455	4977	17648	12648	7294	66664	68392	82608	19768	8912	26000	12359	18464		14148	33392	21680	8909	11424	3667	11785	33512
	Pentacosene : 2	877			540	323	1049	362	519	10161		2794	700		1455	304	481		215	189		295	250		1557	1022
	Pentacosane	4611	1268		822	1474	1998	3444	690	11311	3554	6218	4000	3532	2537	2213	3503		4160	2967	5068	2273	1041	1185	9686	2759
	11 13 Me C25	11875	11809		5063	6079	24112	7247	3793	51992	71976	65536	8523	7450	30144	6888	8886		12354	35768	6018	7548	7018	1272	5529	27424
	5 Me C25	22552	15098		6875	5819	15240	12976	8567	67936	58200	57000	18456	13597	22936	9511	21336		11637	21688	11727	8465	10962	4460	15658	14855
	Heptacosene	4695	2719		1659	1699	6136	2302	773	16231	31512	20080	3642	2047	6870	1730	3488		3336	11962	2500	832	1632	181	1014	8255
Abundance of M+2	Tricosane	430	441				457	614		1002	350	2526	1105	1034	967		850		665	757	1020	1285		552	1382	
	Pentacosene	2109	2838		1484	1723	2155	558	515	5962	8463	11414	2546	2266	3254	1268	2336		1916	4463	2521	2828	1184	1256	1716	3660
	Pentacosene : 2	4742	8355		2490	4479	2640	4179	3156	35640	30672	18808	4160	2354	8518	3084	2605		9591	3040	2788	1463	4009	1947	13282	2724
	Pentacosane	480				475	351	326	278	1739	166		709	1642	499	155	443				290	201	813	250	1576	483
	11 13 Me C25	2416	1620		627	866	4696	2495	883	5167	11150	7196	1298	540	2941	988	1654		1754	3933	414		1359		1136	3574
	5 Me C25	3201	1351		1136	712	192	2154	295	8352	6779	7546	1443	1084	2666	1369	2357		2068	3264	1535	942	1420	381	2696	3195
	Heptacosene	712	730		185		704	294		1494	3328	2447	368	740	861	606	663		390	1988	155		179			965
Percentage of M+1	Tricosane	35.7	24.8		8.5	29.9	22.1	37.5	32.0	24.4	28.0	23.4	18.5	38.9	22.8	30.1	20.4		25.6	14.9	33.4	20.2	18.4	16.8	22.7	31.2
	Pentacosene	30.8	26.7		26.9	19.2	22.9	21.3	31.6	25.4	25.7	29.9	28.9	21.2	25.6	36.2	30.7		25.9	26.4	27.4	25.0	27.6	22.4	30.9	30.1
	Pentacosene : 2	198.9	0.0		77.0	31.4	73.8	35.4	164.2	368.8	0.0	96.0	62.3	0.0	80.2	20.7	44.9		26.7	6.6	0.0	86.3	25.0	0.0	190.3	58.1
	Pentacosane	32.5	20.4		56.1	32.0	24.4	25.4	20.7	28.6	15.6	35.4	34.4	29.3	23.1	28.6	21.9		33.2	23.8	49.9	27.3	12.6	22.1	54.6	21.2
	11 & 13 Me C25	41.1	51.2		72.1	62.2	59.2	36.8	52.8	62.3	46.5	56.1	44.1	60.5	59.6	56.4	61.7		50.1	56.5	52.9	80.2	70.2	67.6	51.0	45.4
	5 Me C25	22.0	21.1		27.0	26.7	21.4	19.9	31.8	21.7	23.4	22.4	21.9	22.1	21.9	20.8	26.3		17.1	23.1	16.7	20.0	23.9	28.4	22.9	18.7
	Heptacosene	28.5	29.6		41.9	47.0	32.7	24.5	26.6	31.0	40.0	32.3	31.9	33.7	30.7	32.6	29.3		29.1	34.5	23.9	19.6	28.6	9.2	21.1	27.4
Percentage of M+2	Tricosane	2.8	5.0		0.0	0.0	2.9	3.9	0.0	1.6	1.0	5.4	6.7	7.0	5.5	0.0	5.1		3.8	3.6	6.2	8.1	0.0	8.8	5.1	0.0
	Pentacosene	2.4	4.7		5.4	6.7	2.8	0.9	2.2	2.3	3.2	4.1	3.7	5.4	3.2	3.7	3.9		3.5	3.5	3.2	7.9	2.9	7.7	4.5	3.3
	Pentacosene : 2	1075.3	511.0		355.2	435.3	185.8	408.5	998.7	1293.6	552.7	646.1	370.1	268.7	469.6	210.2	243.2		1192.9	106.3	214.5	427.8	400.5	614.2	1623.7	154.9
	Pentacosane	3.4	0.0		0.0	10.3	4.3	2.4	8.4	4.4	0.7	0.0	6.1	13.6	4.5	2.0	2.8		0.0	0.0	2.9	2.4	9.8	4.7	8.9	3.7
	11 13 Me C25	8.4	7.0		8.9	8.9	11.5	12.7	12.3	6.2	7.2	6.2	6.7	4.4	5.8	8.1	11.5		7.1	6.2	3.6	0.0	13.6	0.0	10.5	5.9
	5 Me C25	3.1	1.9		4.5	3.3	0.3	3.3	1.1	2.7	2.7	3.0	1.7	1.8	2.5	3.0	2.9		3.0	3.5	2.2	2.2	3.1	2.4	3.9	4.0
	Heptacosene	4.3	7.9		4.7	0.0	3.7	3.1	0.0	2.9	4.2	3.9	3.2	12.2	3.9	11.4	5.6		3.4	5.7	1.5	0.0	3.1	0.0	0.0	3.2

0
0-20
20-40
40-60
60-80
80-100
100+

Figure D.23: Data sheet showing page three of the raw substrate data for the octadec-11-enoic acid-11,12-d_2 fatty acid experiment.

		Sub 1	Sub 2	Sub 3	Sub 4	Sub 5	Sub 6	Sub 7	Sub 8	Sub 9	Sub 10	Sub 11	Sub 12	Sub 13	Sub 14	Sub 15	Sub 16	Sub 17
Abundance of M	Tricosane	5926	7086	5544	13926	6288	16992	18560	5365	18944	10910	2423	5037	2834		9632	12245	15363
	Pentacosene	69944	136256	81840	225024	67520	218944	155584	48776	229760	132544	29496	62840	35968		148480	150208	224384
	Pentacosene : 2	5968	6146	3733	7587	2745	7120	4747	2233	14948	2138	1512	470	920		2461	1484	1037
	Pentacosane	2368	4132	2577	5458	3005	6836	5861	29550	7592	3952	767	1443	1354		4321	4410	7654
	11 13 Me C25	11505	32960	13462	41256	10920	36440	17968	5974	34032	15775	3737	8816	6084		17344	16640	36536
	5 Me C25	59744	108120	55600	157568	43464	145664	107056	31152	153792	98632	19760	38272	22544		109648	104640	143296
	Heptacosene	10717	20880	11945	36456	9079	40744	22720	5067	31144	17960	4035	9426	3750		22808	18464	31880
Abundance of M+1	Tricosane	1727	1867	1967	4027	1514	3687	5636	1362	4201	2957	380	1165	1192		3146	2856	4702
	Pentacosene	9616	37192	23384	59576	17728	58544	39488	10941	57560	37144	8990	16019	11172		46944	40000	65304
	Pentacosene : 2	883	1715	1179	2461	1264	2079	1326	619	2822	194	341	158	429		1093	972	561
	Pentacosane	1081	1209	385	1990	986	1385	2091	796	2398	837	413	403	374		1248	2105	1524
	11 13 Me C25	6280	15548	6234	22808	4119	15855	9648	2641	15955	8969	2200	4285	3444		10737	9942	20432
	5 Me C25	12637	21544	12035	36296	8766	33992	23248	6457	31592	18984	5074	8351	4975		18792	25088	31560
	Heptacosene	2744	5111	2641	9153	2800	11619	8001	2023	9121	5619	854	2822	1191		7397	6587	11014
Abundance of M+2	Tricosane		404	272	685	371	929	694	183	804	629			566		370	725	747
	Pentacosene	2138	3808	2665	8935	2279	7951	6509	2567	6815	4431	750	2963	1230		6146	1021	8034
	Pentacosene : 2		617		410	259	180	330		679						276		
	Pentacosane				208	219	291		201	508	370						177	159
	11 13 Me C25	1480	1537	776	3835	1194	2791	1549	213	2534	1595	517	1108	370		1658	1017	3179
	5 Me C25		2308	1074	3049	848	3132	2881	614	4406	1886	817	1134	1030		3297	3506	3136
	Heptacosene	625	1089	299	2257	337	1657	1359	811	1883	962	292	586	219		1602	1229	1326
Percentage of M+1	Tricosane	29.1	26.3	35.5	28.9	24.1	21.7	30.4	25.4	22.2	27.1	15.7	23.1	42.1		32.7	23.3	30.6
	Pentacosene	13.7	27.3	28.6	26.5	26.3	26.7	25.4	22.4	25.1	28.0	30.5	25.5	31.1		31.6	26.6	29.1
	Pentacosene : 2	14.8	27.9	31.6	32.4	46.0	29.2	27.9	27.7	18.9	9.1	22.6	33.6	46.6		44.4	65.5	54.1
	Pentacosane	45.7	29.3	14.9	36.5	32.8	20.3	35.7	2.7	31.6	21.2	53.8	27.9	27.6		28.9	47.7	19.9
	11 & 13 Me C25	54.6	47.2	46.3	55.3	37.7	43.5	53.7	44.2	46.9	56.9	58.9	48.6	56.6		61.9	59.7	55.9
	5 Me C25	21.2	19.9	21.6	23.0	20.2	23.3	21.7	20.7	20.5	19.2	25.7	21.8	22.1		17.1	24.0	22.0
	Heptacosene	25.6	24.5	22.1	25.1	30.8	28.5	35.2	39.9	29.3	31.3	21.2	29.9	31.8		32.4	35.7	34.5
Percentage of M+2	Tricosane	0.0	5.7	4.9	4.9	5.9	5.5	3.7	3.4	4.2	5.8	0.0	0.0	20.0		3.8	5.9	4.9
	Pentacosene	3.1	2.8	3.3	4.0	3.4	3.6	4.2	5.3	3.0	3.3	2.5	4.7	3.4		4.1	0.7	3.6
	Pentacosene : 2	0.0	10.0	0.0	5.4	9.4	2.5	7.0	0.0	4.5	0.0	0.0	0.0	0.0		11.2	0.0	0.0
	Pentacosane	0.0	0.0	0.0	3.8	7.3	4.3	0.0	0.7	6.7	9.4	0.0	0.0	0.0		0.0	4.0	2.1
	11 13 Me C25	12.9	4.7	5.8	9.3	10.9	7.7	8.6	3.6	7.4	10.1	13.8	12.6	6.1		9.6	6.1	8.7
	5 Me C25	0.0	2.1	1.9	1.9	2.0	2.2	2.7	2.0	2.9	1.9	4.1	3.0	4.6		3.0	3.4	2.2
	Heptacosene	5.8	5.2	2.5	6.2	3.7	4.1	6.0	16.0	6.0	5.4	7.2	6.2	5.8		7.0	6.7	4.2

0
0-20
20-40
40-60
60-80
80-100
100+

Figure D.24: Data sheet showing page one of the raw substrate data for the eicos-9-enoic acid-9,10-d_2 fatty acid experiment.

		Sub 18	Sub 19	Sub 20	Sub 21	Sub 22	Sub 23	Sub 24	Sub 25	Sub 26	Sub 27	Sub 28	Sub 29	Sub 30	Sub 31	Sub 32	Sub 33	Sub 34
Abundance of M	Tricosane	26496	21568	5388	22608	14751	14090	11481	9434	25504	19688	11824	10824	13915	4342	16202	12568	17984
	Pentacosene	416256	367808	68600	367424	141568	208768	156096	163648	204032	219520	150464	1216464	172608	82920	206336	127816	275968
	Pentacosene : 2	2060	2416	1628	2350	2263	1515	1325	1049	2202	931	2612	2997	2364	1165	1546	1431	1992
	Pentacosane	9881	6508	1923	11149	3302	8804	4407	3435	11032	6535	5999	5218	4807	2559	8092	5202	8439
	11 13 Me C25	58024	52080	9078	47720	16424	32968	13863	18536	26120	25744	14752	11425	21328	9306	22432	16233	35864
	5 Me C25	254336	213376	49488	244928	95808	148032	92552	112744	139584	140864	92088	87872	122504	67368	143424	78568	184832
	Heptacosene	55232	46752	11030	50312	18504	28592	21232	21824	26768	31368	20152	17056	23904	9350	30656	16944	45360
Abundance of M+1	Tricosane	7557	5200	1308	6016	3195	3509	3406	3372	6040	6047	3704	2829	2986	1634	3703	2479	5359
	Pentacosene	116648	97288	22480	96568	39216	56288	40048	45120	54024	54080	41880	32768	49992	22000	63736	34432	75008
	Pentacosene : 2	463	677	708	359	471	376	647	251	718	403	697	773	645	215	522	747	214
	Pentacosane	3517	2582	663	2610	1372	1802	1110	1011	2250	2204	2030	1911	2380	429	3040	994	1828
	11 13 Me C25	25768	25312	2941	26944	8394	19192	6718	11953	13805	12896	9518	6473	9848	4853	10509	7476	17384
	5 Me C25	59848	48752	10118	53592	20136	30400	22944	25280	31688	30824	22472	22360	27376	14152	30928	17488	41312
	Heptacosene	15348	13553	2504	14924	6401	9965	6214	7205	9203	10283	5101	5761	7145	3076	8304	4431	10861
Abundance of M+2	Tricosane	1022	760		870	488	233		426	811	472	527	316	571		914	269	587
	Pentacosene	14380	12482	3523	13566	5038	6326	6144	6684	7028	6923	7670	5104	6144	4360	6992	5744	9736
	Pentacosene : 2					317							199	229				
	Pentacosane	270	359		349		286			334	838	206	276		155		275	229
	11 13 Me C25	2905	2708	971	2635	1832	3079	1234	1704	1680	1369	1020	1040	1558		164	1025	2601
	5 Me C25	6448	5586	1701	5534	2355	3974	2370	3243	3021	3322	2058	1625	2413	1628	3177	2590	4506
	Heptacosene	4255	1363	206	2370	953	1157	719	1379	2136	1204	1246	1692	1088	376	923	1028	2743
Percentage of M+1	Tricosane	28.5	24.1	24.3	26.6	21.7	24.9	29.7	35.7	23.7	30.7	31.3	26.1	21.5	37.6	22.9	19.7	29.8
	Pentacosene	28.0	26.5	32.8	26.3	27.7	27.0	25.7	27.6	26.5	24.6	27.8	2.7	29.0	26.5	30.9	26.9	27.2
	Pentacosene : 2	22.5	28.0	43.5	15.3	20.8	24.8	48.8	23.9	32.6	43.3	26.7	25.8	27.3	18.5	33.8	52.2	10.7
	Pentacosane	35.6	39.7	34.5	23.4	41.6	20.5	25.2	29.4	20.4	33.7	33.8	36.6	49.5	16.8	37.6	19.1	21.7
	11 & 13 Me C25	44.4	48.6	32.4	56.5	51.1	58.2	48.5	64.5	52.9	50.1	64.5	56.7	46.2	52.1	46.8	46.1	48.5
	5 Me C25	23.5	22.8	20.4	21.9	21.0	20.5	24.8	22.4	22.7	21.9	24.4	25.4	22.3	21.0	21.6	22.3	22.4
	Heptacosene	27.8	29.0	22.7	29.7	34.6	34.9	29.3	33.0	34.4	32.8	25.3	33.8	29.9	32.9	27.1	26.2	23.9
Percentage of M+2	Tricosane	3.9	3.5	0.0	3.8	3.3	1.7	0.0	4.5	3.2	2.4	4.5	2.9	4.1	0.0	5.6	2.1	3.3
	Pentacosene	3.5	3.4	5.1	3.7	3.6	3.0	3.9	4.1	3.4	3.2	5.1	0.4	3.6	5.3	3.4	4.5	3.5
	Pentacosene : 2	0.0	0.0	0.0	0.0	14.0	0.0	0.0	0.0	0.0	0.0	0.0	6.6	9.7	0.0	0.0	0.0	0.0
	Pentacosane	2.7	5.5	0.0	3.1	0.0	3.2	0.0	0.0	3.0	12.8	3.4	5.3	0.0	6.1	0.0	5.3	2.7
	11 13 Me C25	5.0	5.2	10.7	5.5	11.2	9.3	8.9	9.2	6.4	5.3	6.9	9.1	7.3	0.0	0.7	6.3	7.3
	5 Me C25	2.5	2.6	3.4	2.3	2.5	2.7	2.6	2.9	2.2	2.4	2.2	1.8	2.0	2.4	2.2	3.3	2.4
	Heptacosene	7.7	2.9	1.9	4.7	5.2	4.0	3.4	6.3	8.0	3.8	6.2	9.9	4.6	4.0	3.0	6.1	6.0

0
0-20
20-40
40-60
60-80
80-100
100+

Figure D.25: Data sheet showing page two of the raw substrate data for the eicos-9-enoic acid-9,10-d_2 fatty acid experiment.

		Sub 1	Sub 2	Sub 3	Sub 4	Sub 5	Sub 6	Sub 7	Sub 8	Sub 9	Sub 10	Sub 11	Sub 12	Sub 13	Sub 14	Sub 15	Sub 16	Sub 17	Sub 18	Sub 19	Sub 20	Sub 21	Sub 22	Sub 23	Sub 24	Sub 25	Sub 26
Abundance of M	Tricosane	7276	25984	6955	17496	31728	9075	15337	24672	5542	9524	4323	8798	24104	1568	2139	10201	14121	7095	7613	5469		16155	5586	10333	7110	24592
	Pentacosene	90648	408064	106592	198656	398464	89328	235264	234368	49720	82192	31408	87592	426688	17936	27872	129592	151040	153792	80552	60576		146368	734880	178240	93184	380160
	Pentacosene : 2	2234	45032	9424	11068	19104	6321	9593	19424	1045	4990	870	2183	2181	1196	557	1370	2984	1530	450	1103		557	1629	1864	950	3097
	Pentacosane	4985	10683	3487	8885	10385	5655	8215	9970	2596	4292	1858	3740	11424	634	969	4557	7646	3525	3369	1491		6304	3237	4006	2413	10036
	11 13 Me C25	12486	58520	14310	27592	63672	7545	31360	31368	4804	10193	4857	9726	52448	3314	2840	17488	19680	22424	10155	9324		21584	10473	28888	17176	563240
	5 Me C25	91760	321472	72056	160320	306304	9905	182144	163840	36368	68048	27088	63072	316096	14412	18864	94512	116544	113184	53176	44576		96800	54416	135808	63096	270912
	Heptacosene	16712	64048	16097	38752	57320	13931	38192	37984	8134	14519	5478	8633	61368	2569	2933	16096	23160	17672	9876	9659		19576	12175	26296	13253	53976
Abundance of M+1	Tricosane	1150	4820	1139	3882	7018	2714	4577	6105	1477	2266	1108	1834	7479	515	358	2700	3775	2260	1609	1848		4274	942	3304	2278	6900
	Pentacosene	22040	100688	25072	51112	106424	20928	57304	68104	13698	21112	9486	23712	112040	4252	7140	36760	38160	39432	22048	17480		40272	19808	44768	28608	101032
	Pentacosene : 2	968	9919	2040	2022	4243	2234	2476	6864	224	2630	166	836	601	616	256	623	541	327	217	426		333	452	359	232	1302
	Pentacosane	1310	4805	1232	2509	1795		2088	4022	931	755	589	751	2788	339	455	1169	1615	1059	744	379		2609	838	1244	1183	1981
	11 13 Me C25	7486	29184	6138	12998	31904	1613	16768	15998	4183	7351	3177	3185	25808	1622	2353	7526	12296	11411	4421	4949		12905	7280	15438	8379	30256
	5 Me C25	16960	66968	17960	34928	64224	5729	42808	36776	8583	14892	6444	16353	63608	3998	4153	21208	24552	21712	9982	2790		19040	12432	30272	15564	59192
	Heptacosene	5616	17824	4594	10002	20576	4396	12118	11956	2214	4734	1298	3282	19656	930	861	5180	6861	7309	3600	0		6442	3446	7806	3513	17096
Abundance of M+2	Tricosane	457	576	399	811	1032	398	688	1031		263			1312		193	190	478	275	368	191		585		467	274	1359
	Pentacosene	3314	12394	3637	6288	11976	2695	6944	8182	2048	3193	1028	3505	14700	1138	878	3589	6005	4903	2912	2970		5468	2956	5755	2987	12875
	Pentacosene : 2		2690	980	611	1001	1371	619	1616	166	762			190				206									
	Pentacosane	376	272	503	205			814	1027	158		154		272			317		382	219	278		248	617	152		549
	11 13 Me C25	802	4352	1310	2497	5217	336	2522	2544	506	1318	491	618	4083	251	327	1302	1586	1231	834	954		1625	1508	1995	1350	3657
	5 Me C25	1727	6457	1785	3944	6580	941	2868	4151	1571	2263	1235	871	7222	587	456	2574	2837	2248	1155	1076		2571	1465	4051	1518	8583
	Heptacosene	1210	2804	590	1109	2617	546	1322	1543	188	327	248	653	2091	227	334	360	922	1282		309		1192	368	1077	533	1843
Percentage of M+1	Tricosane	15.8	18.5	16.4	22.2	22.1	29.9	29.8	24.7	26.7	23.8	25.6	20.8	31.0	32.8	16.7	26.5	26.7	31.9	21.1	33.8		26.5	16.9	32.0	32.0	28.1
	Pentacosene	24.3	24.7	23.5	25.7	26.7	23.4	24.4	29.1	27.6	25.7	30.2	27.1	26.3	23.7	25.6	28.4	25.3	25.6	27.4	28.9		27.5	2.7	25.1	30.7	26.6
	Pentacosene : 2	43.3	22.0	21.6	18.3	22.2	35.3	25.8	35.3	21.4	52.7	19.1	38.3	27.6	51.5	46.0	45.5	18.1	21.4	48.2	38.6		59.8	27.7	19.3	24.4	42.0
	Pentacosane	26.3	45.0	35.3	28.2	17.3	0.0	25.4	40.3	35.9	17.6	31.7	20.1	24.4	53.5	47.0	25.7	21.1	30.0	22.1	25.4		41.4	25.9	31.1	49.0	19.7
	11 & 13 Me C25	60.0	49.9	42.9	47.1	50.1	21.4	53.5	51.0	87.1	72.1	65.4	32.7	49.2	48.9	82.9	43.0	62.5	50.9	43.5	53.1		59.8	69.5	53.4	48.8	5.4
	5 Me C25	18.5	20.8	24.9	21.8	21.0	57.8	23.5	22.4	23.6	21.9	23.8	25.9	20.1	27.7	22.0	22.4	21.1	19.2	18.8	6.3		19.7	22.8	22.3	24.7	21.8
	Heptacosene	33.6	27.8	28.5	25.8	35.9	31.6	31.7	31.5	27.2	32.6	23.7	38.0	32.0	36.2	29.4	32.2	29.6	41.4	36.5	0.0		32.9	28.3	29.7	26.5	31.7
Percentage of M+2	Tricosane	6.3	2.2	5.7	4.6	3.3	4.4	4.5	4.2	0.0	2.8	0.0	0.0	5.4	0.0	9.0	1.9	3.4	3.9	4.8	3.5		3.6	0.0	4.5	3.9	5.5
	Pentacosene	3.7	3.0	3.4	3.2	3.0	3.0	3.0	3.5	4.1	3.9	3.3	4.0	3.4	6.3	3.2	2.8	4.0	3.2	3.6	4.9		3.7	0.4	3.2	3.2	3.4
	Pentacosene : 2	0.0	6.0	10.4	5.5	5.2	21.7	6.5	8.3	15.9	15.3	0.0	0.0	8.7	0.0	0.0	0.0	6.9	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0
	Pentacosane	7.5	2.5	14.4	2.3	0.0	0.0	9.9	10.3	6.1	0.0	8.3	0.0	2.4	0.0	0.0	7.0	0.0	10.8	6.5	18.6		3.9	19.1	3.8	0.0	5.5
	11 13 Me C25	6.4	7.4	9.2	9.0	8.2	4.5	8.0	8.1	10.5	12.9	10.1	6.4	7.8	7.6	11.5	7.4	8.1	5.5	8.2	10.2		7.5	14.4	6.9	7.9	0.6
	5 Me C25	1.9	2.0	2.5	2.5	2.1	9.5	1.6	2.5	4.3	3.3	4.6	1.4	2.3	4.1	2.4	2.7	2.4	2.0	2.2	2.4		2.7	2.7	3.0	2.4	3.2
	Heptacosene	7.2	4.4	3.7	2.9	4.6	3.9	3.5	4.1	2.3	2.3	4.5	7.6	3.4	8.8	11.4	2.2	4.0	7.3	0.0	3.2		6.1	3.0	4.1	4.0	3.4

0
0-20
20-40
40-60
60-80
80-100
100+

Figure D.26: Data sheet showing page one of the raw substrate data for the eicos-11-enoic acid-11,12-d_2 fatty acid experiment.

		Sub 27	Sub 28	Sub 29	Sub 30	Sub 31	Sub 32	Sub 33	Sub 34	Sub 35	Sub 36	Sub 37	Sub 38	Sub 39	Sub 40	Sub 41	Sub 42	Sub 43	Sub 44	Sub 45	Sub 46	Sub 47	Sub 48	Sub 49	Sub 50	Sub 51	Sub 52	Sub 53
Abundance of M	Tricosane	13249	10092	10690	10580	2899	4232	8052	6347	8846	13323	13017	14283	5654	17856	11434		15084	9595	17344	16106	19224	10586	16640	22456	9561	13756	10854
	Pentacosene	133888	138880	130560	146624	45088	77400	77872	110944	91336	253824	226304	145152	90200	314176	185728		237760	142912	173376	256768	207296	173376	178368	239232	142016	203840	159680
	Pentacosene : 2	1922	1487	1106	1306	1114	576	990	864	912	1142	2818	1172	1076	2199	705		1509	1832	1912	1241	1440	1716	1589	1960	1977	1427	2351
	Pentacosane	6443	3035	4438	4161	1907	1400	3117	1898	2687	6384	8567	3687	3000	6819	3211		4793	5538	5279	7185	10216	4755	6395	8324	3598	4272	4610
	11 13 Me C25	16768	18000	17464	26760	5701	10051	9883	13568	9212	34792	25632	15097	6852	40880	27336		24696	22592	18008	39056	27224	24440	22600	23088	20056	19968	22304
	5 Me C25	107112	95488	96120	111024	32496	59560	57624	86808	59752	216192	169984	105792	60952	232000	139264		169920	113344	119656	168064	148096	142848	125232	142272	122888	140864	117944
	Heptacosene	21744	22296	17616	19832	6298	10364	9219	17472	11182	32216	30456	18984	13512	45784	22320		33912	21648	25224	37024	30608	24216	26080	26160	22776	24688	22152
Abundance of M+1	Tricosane	3725	1836	2211	2853	960	1041	1628	2028	2584	3305	4898	3022	1904	4569	3041		3322	2788	4540	4402	5129	3410	4161	7546	3426	3154	2506
	Pentacosene	34664	34960	33600	39016	11458	20808	21912	31776	22584	63936	56296	42560	25760	74264	48920		61080	41088	45408	68000	56184	50992	48672	63656	36608	55848	47488
	Pentacosene : 2	796	381	240	410	387	161	164	324	260	444	335	491	303	745	528		192	223	397	616	270	684	920	729	452	200	583
	Pentacosane	2114	1128	1952	1307	753	470	1089	877	949	1878	2581	1351	466	2005	1741		2994	1150	1725	2115	2561	978	1449	3266	1168	1619	1463
	11 13 Me C25	13401	10085	8358	17000	3154	5587	5898	9130	5029	18816	15899	7735	4510	25064	16250		12169	10870	7712	18760	12504	14720	9818	13745	7452	11800	9236
	5 Me C25	23968	21720	21824	22576	9034	14858	10998	16345	10824	42104	33656	25576	12801	51360	30352		36168	24488	27176	37144	34296	30256	29872	31632	25152	29832	26568
	Heptacosene	5157	5376	4629	6300	2163	2828	2456	4390	3739	9654	9979	4368	3728	13155	6734		11640	4212	7409	10940	9390	5981	8832	7406	6445	9579	6767
Abundance of M+2	Tricosane	786	212	669	391	329	195		183	316	333	324	317	199	371	1009		792	438	627	338	784	313	886	1139	327	424	192
	Pentacosene	5245	4602	6639	4464	1917	2439	2371	5217	2929	8097	7213	4281	2663	8776	6270		9433	4679	5069	7957	7558	6976	7634	7915	4669	6518	5944
	Pentacosene : 2	656				309						478				207				266	184							
	Pentacosane					170						324		376	324	164				957		705	450	403	334			
	11 13 Me C25	1528	2305	606	2022	242	327	617	1183	823	1531	2156	2653	536	2576	1214		1910	1360	2365	2271	1216	3077	1684	1958	931	1821	1700
	5 Me C25	3328	1840	2012	2603	934	1327	2104	3185	1482	6420	3690	3952	1187	3600	2915		3680	1871	4119	4671	2962	3655	2910	3452	3087	1773	2932
	Heptacosene	1517	1473	876	1146	271	421	218	449	607	1482	1729	1349	793	1333	807		2194	924	1391	2193	1472	1203	1245	1252	2000		991
Percentage of M+1	Tricosane	28.1	18.2	20.7	27.0	33.1	24.6	20.2	32.0	29.2	24.8	37.6	21.2	33.7	25.6	26.6		22.0	29.1	26.2	27.3	26.7	32.2	25.0	33.6	35.8	22.9	23.1
	Pentacosene	25.9	25.2	25.7	26.6	25.4	26.9	28.1	28.6	24.7	25.2	24.9	29.3	28.6	23.6	26.3		25.7	28.8	26.2	26.5	27.1	29.4	27.3	26.6	25.8	27.4	29.7
	Pentacosene : 2	41.4	25.6	21.7	31.4	34.7	28.0	16.6	37.5	28.5	38.9	11.9	41.9	28.2	33.9	74.9		12.7	12.2	20.8	49.6	18.8	39.9	57.9	37.2	22.9	14.0	24.8
	Pentacosane	32.8	37.2	44.0	31.4	39.5	33.6	34.9	46.2	35.3	29.4	30.1	36.6	15.5	29.4	54.2		62.5	20.8	32.7	29.4	25.1	20.6	22.7	39.2	32.5	37.9	31.7
	11 & 13 Me C25	79.9	56.0	47.9	63.5	55.3	55.6	59.7	67.3	54.6	54.1	62.0	51.2	65.8	61.3	59.4		49.3	48.1	42.8	48.0	45.9	60.2	43.4	59.5	37.2	59.1	41.4
	5 Me C25	22.4	22.7	22.7	20.3	27.8	24.9	19.1	18.8	18.1	19.5	19.8	24.2	21.0	22.1	21.8		21.3	21.6	22.7	22.1	23.2	21.2	23.9	22.2	20.5	21.2	22.5
	Heptacosene	23.7	24.1	26.3	31.8	34.3	27.3	26.6	25.1	33.4	30.0	32.8	23.0	27.6	28.7	30.2		34.3	19.5	29.4	29.5	30.7	24.7	33.9	28.3	28.3	38.8	30.5
Percentage of M+2	Tricosane	5.9	2.1	6.3	3.7	11.3	4.6	0.0	2.9	3.6	2.5	2.5	2.2	3.5	2.1	8.8		5.3	4.6	3.6	2.1	4.1	3.0	5.3	5.1	3.4	3.1	1.8
	Pentacosene	3.9	3.3	5.1	3.0	4.3	3.2	3.0	4.7	3.2	3.2	3.2	2.9	3.0	2.8	3.4		4.0	3.3	2.9	3.1	3.6	4.0	4.3	3.3	3.3	3.2	3.7
	Pentacosene : 2	34.1	0.0	0.0	0.0	27.7	0.0	0.0	0.0	0.0	0.0	17.0	0.0	0.0	0.0	29.4		0.0	0.0	13.9	14.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Pentacosane	0.0	0.0	0.0	0.0	8.9	0.0	0.0	0.0	0.0	0.0	3.8	0.0	12.5	4.8	5.1		0.0	0.0	18.1	0.0	6.9	9.5	6.3	4.0	0.0	0.0	0.0
	11 13 Me C25	9.1	12.8	3.5	7.6	4.2	3.3	6.2	8.7	8.9	4.4	8.4	17.6	7.8	6.3	4.4		7.7	6.0	13.1	5.8	4.5	12.6	7.5	8.5	4.6	9.1	7.6
	5 Me C25	3.1	1.9	2.1	2.3	2.9	2.2	3.7	3.7	2.5	3.0	2.2	3.7	1.9	1.6	2.1		2.2	1.7	3.4	2.8	2.0	2.6	2.3	2.4	2.5	1.3	2.5
	Heptacosene	7.0	6.6	5.0	5.8	4.3	4.1	2.4	2.6	5.4	4.6	5.7	7.1	5.9	2.9	3.6		6.5	4.3	5.5	5.9	4.8	5.0	4.8	4.8	8.8	0.0	4.5

0
0-20
20-40
40-60
60-80
80-100
100+

Figure D.27: Data sheet showing page two of the raw substrate data for the eicos-11-enoic acid-11,12-d_2 fatty acid experiment.

Appendix E

Excel data sheets of the raw data for Chapter 7

The following pages show the raw data sheets from the chapter exploring the relationship between substrate incorporation and the amount of food consumed. The cells are colour coded according to the percentage abundance calculated from the raw mass spectra data, see Section 3.4.2.1. Please note that some of the data extends across several pages and some of the repeats failed, therefore the data is not presented. The mean value for the group is shown at the end of the data and the lowest and highest values are colour coded in red and green respectively.

A study into the relationship between substrate incorporation and amount of food consumed

Figure E.1-E.2: Raw data showing repeat one of the basic yellow data for *Formica lemani* at a dye concentration of 1 g/L

Figure E.3-E.4: Raw data showing repeat two of the basic yellow data for *Formica lemani* at a dye concentration of 1 g/L

Figure E.5-E.6: Raw data showing repeat three of the basic yellow data for *Formica lemani* at a dye concentration of 1 g/L

Figure E.7-E.8: Raw data showing repeat one of the basic yellow data for *Formica lemani* at a dye concentration of 2 g/L

Figure E.9-E.10: Raw data showing repeat two of the basic yellow data for *Formica lemani* at a dye concentration of 2 g/L

Figure E.11-E.12: Raw data showing repeat three of the basic yellow data for *Formica lemani* at a dye concentration of 2 g/L

Figure E.13-E.14: Raw data showing repeat one of the basic yellow data for *Formica lemani* at a dye concentration of 5 g/L

Figure E.15-E.16: Raw data showing repeat three of the basic yellow data for *Formica lemani* at a dye concentration of 5 g/L

Figure E.17-E.18: Raw data showing the basic yellow dye only control for *Formica lemani*

Figure E.19: Raw data showing the sodium [$^{13}C_2$]acetate control for *Formica lemani*

Figure E.20: Raw data showing repeat one of the basic yellow data for *Myrmica rubra* at a dye concentration of 1 g/L

Figure E.21: Raw data showing repeat two of the basic yellow data for *Myrmica rubra* at a dye concentration of 1 g/L

Figure E.22: Raw data showing repeat one of the basic yellow data for *Myrmica rubra* at a dye concentration of 2 g/L

Figure E.23: Raw data showing repeat two of the basic yellow data for *Myrmica rubra* at a dye concentration of 2 g/L

Figure E.24-E.25: Raw data showing repeat three of the basic yellow data for *Myrmica rubra* at a dye concentration of 2 g/L

Figure E.26: Raw data showing repeat one of the basic yellow data for *Myrmica rubra* at a dye concentration of 5 g/L

Figure E.27-E.28: Raw data showing repeat two of the basic yellow data for *Myrmica rubra* at a dye concentration of 5 g/L

Figure E.29-E.30: Raw data showing repeat three of the basic yellow data for *Myrmica rubra* at a dye concentration of 5 g/L

Figure E.31: Raw data showing the basic yellow dye only control for *Myrmica rubra*

Figure E.32: Raw data showing the sodium [$^{13}C_2$]acetate control for *Myrmica rubra*

Figure E.33: Raw data showing repeat one of the rhodamine B data for *Formica fusca* at a dye concentration of 2 g/L

Figure E.34: Raw data showing repeat two of the rhodamine B data for *Formica fusca* at a dye concentration of 2 g/L

Figure E.35: Raw data showing repeat three of the rhodamine B data for *Formica fusca* at a dye concentration of 2 g/L

Figure E.36: Raw data showing the rhodamine B dye only control for *Formica fusca*

Figure E.38: Raw data showing repeat one of the rhodamine B data for *Myrmica rubra* at a dye concentration of 1 g/L

Figure E.39: Raw data showing repeat two of the rhodamine B data for *Myrmica rubra* at a dye concentration of 1 g/L

Figure E.39: Raw data showing repeat three of the rhodamine B data for *Myrmica rubra* at a dye concentration of 1 g/L

Figure E.41: Raw data showing repeat two of the rhodamine B data for *Myrmica rubra* at a dye concentration of 2 g/L

Figure E.42: Raw data showing repeat three of the rhodamine B data for *Myrmica rubra* at a dye concentration of 2 g/L

Figure E.43: Raw data showing repeat one of the rhodamine B data for *Myrmica rubra* at a dye concentration of 5 g/L

Figure E.44: Raw data showing repeat two of the rhodamine B data for *Myrmica rubra* at a dye concentration of 5 g/L

Figure E.45: Raw data showing repeat three of the rhodamine B data for *Myrmica rubra* at a dye concentration of 5 g/L

Figure E.46: Raw data showing the rhodamine B dye only control for *Myrmica rubra*

Basic yellow 1g / L Repeat 1		*Formica lemani*														
		YF_11_1	YF_11_2	YF_11_3	YF_11_4	YF_11_5	YF_11_6	YF_11_7	YF_11_8	YF_11_9	YF_11_10	YF_11_11	YF_11_12	YF_11_13	YF_11_14	YF_11_15
Abundance of M	Pentacosene	222144	174144	231936	196160	274560	253760	204800	204352	265984	276160	229440	261184	267520	280512	258304
	Heptacosene	72168	52280	82792	69600	150848	98656	60728	58352	107640	122840	83024	94368	100248	141824	106608
Abundance of M+1	Pentacosene	76856	58640	78040	65392	91736	86880	68320	68672	87216	87248	76400	86080	90632	94440	85416
	Heptacosene	32248	23032	34528	29656	64088	41832	24976	26120	46792	50712	34016	40880	43584	59912	44712
Abundance of M+2	Pentacosene	16800	12457	16456	14240	18944	18304	14459	14974	18272	17992	16984	18336	18576	20720	18944
	Heptacosene	10462	6164	9835	8308	19408	11456	7327	7785	13451	14104	9924	13240	12528	17232	12589
Percentage of M+1	Pentacosene	34.6	33.7	33.6	33.3	33.4	34.2	33.4	33.6	32.8	31.6	33.3	33.0	33.9	33.7	33.1
	Heptacosene	44.7	44.1	41.7	42.6	42.5	42.4	41.1	44.8	43.5	41.3	41.0	43.3	43.5	42.2	41.9
Percentage of M+2	Pentacosene	7.6	7.2	7.1	7.3	6.9	7.2	7.1	7.3	6.9	6.5	7.4	7.0	6.9	7.4	7.3
	Heptacosene	14.5	11.8	11.9	11.9	12.9	11.6	12.1	13.3	12.5	11.5	12.0	14.0	12.5	12.2	11.8
Average M+n pentacosene and heptacosene values		25.3	24.2	23.6	23.8	23.9	23.9	23.4	24.8	23.9	22.7	23.4	24.3	24.2	23.9	23.5

0
0-20
20-40
40-60
60-80
80-100
100+

Figure E.1: Data sheet showing page one, repeat one of the basic yellow data for *Formica lemani* at a dye concentration of 1 g/L.

Basic yellow 1g / L Repeat 1		*Formica lemani*														
		YF_11_16	YF_11_17	YF_11_18	YF_11_19	YF_11_20	YF_11_21	YF_11_22	YF_11_23	YF_11_24	YF_11_25	YF_11_26	YF_11_27	YF_11_28	YF_11_29	YF_11_30
Abundance of M	Pentacosene	262912	275648	284096	266304	258240	274496	282496	275200	263488	294144	273664	295424	287232	295616	237312
	Heptacosene	97824	116832	147200	100344	107344	116296	183168	117584	103344	168000	119976	151552	115720	134656	75880
Abundance of M+1	Pentacosene	86680	93592	94832	89152	86456	93584	94336	93128	88584	98080	91632	98752	94832	98560	81176
	Heptacosene	41512	51504	61840	43136	47112	48088	77664	49192	45144	67064	50264	64512	49224	55952	35008
Abundance of M+2	Pentacosene	18336	19200	19416	17992	17264	18600	19136	18752	18984	19912	19464	20312	19008	20264	18480
	Heptacosene	13042	15091	18024	12507	13325	13623	22048	14467	13380	19320	14781	19008	13891	16271	10810
Percentage of M+1	Pentacosene	33.0	34.0	33.4	33.5	33.5	34.1	33.4	33.8	33.6	33.3	33.5	33.4	33.0	33.3	34.2
	Heptacosene	42.4	44.1	42.0	43.0	43.9	41.3	42.4	41.8	43.7	39.9	41.9	42.6	42.5	41.6	46.1
Percentage of M+2	Pentacosene	7.0	7.0	6.8	6.8	6.7	6.8	6.8	6.8	7.2	6.8	7.1	6.9	6.6	6.9	7.8
	Heptacosene	13.3	12.9	12.2	12.5	12.4	11.7	12.0	12.3	12.9	11.5	12.3	12.5	12.0	12.1	14.2
Average M+n pentacosene and heptacosene values		23.9	24.5	23.6	23.9	24.1	23.5	23.7	23.7	24.4	22.9	23.7	23.9	23.5	23.5	25.6

0
0-20
20-40
40-60
60-80
80-100
100+
23.9%

Figure E.2: Data sheet showing page two, repeat one of the basic yellow data for *Formica lemani* at a dye concentration of 1 g/L.

Basic yellow 1g / L Repeat 2		*Formica lemani*														
		YF_12_1	YF_12_2	YF_12_3	YF_12_4	YF_12_5	YF_12_6	YF_12_7	YF_12_8	YF_12_9	YF_12_10	YF_12_11	YF_12_12	YF_12_13	YF_12_14	YF_12_15
Abundance of M	Pentacosene	157952	222912	226624	289280	236544	305856	232768	292096	196672	311040	298048	309184	305920	302208	234368
	Heptacosene	49944	73392	81680	113976	82040	170496	76576	123352	63552	155584	202560	154048	161728	182016	89616
Abundance of M+1	Pentacosene	55024	74296	78808	96944	81960	103640	79368	99240	65104	103104	99960	104912	106184	107208	79296
	Heptacosene	23096	32912	35312	49560	34776	71000	32552	52656	26600	64088	84888	66768	69440	78416	38344
Abundance of M+2	Pentacosene	12758	14817	16952	20664	17336	22840	17080	21056	14269	20536	21056	22728	22216	23336	17144
	Heptacosene	6732	9355	10986	3837	9528	20664	9454	13940	7206	18144	24328	18504	20672	23856	12046
Percentage of M+1	Pentacosene	34.8	33.3	34.8	33.5	34.6	33.9	34.1	34.0	33.1	33.1	33.5	33.9	34.7	35.5	33.8
	Heptacosene	46.2	44.8	43.2	43.5	42.4	41.6	42.5	42.7	41.9	41.2	41.9	43.3	42.9	43.1	42.8
Percentage of M+2	Pentacosene	8.1	6.6	7.5	7.1	7.3	7.5	7.3	7.2	7.3	6.6	7.1	7.4	7.3	7.7	7.3
	Heptacosene	13.5	12.7	13.5	3.4	11.6	12.1	12.3	11.3	11.3	11.7	12.0	12.0	12.8	13.1	13.4
Average M+n pentacosene and heptacosene values		25.7	24.4	24.7	21.9	24.0	23.8	24.1	23.8	23.4	23.2	23.6	24.2	24.4	24.8	24.3

0
0-20
20-40
40-60
60-80
80-100
100+

Figure E.3: Data sheet showing page one, repeat two of the basic yellow data for *Formica lemani* at a dye concentration of 1 g/L.

Basic yellow 1g / L Repeat 2		*Formica lemani*														
		YF_12_16	YF_12_17	YF_12_18	YF_12_19	YF_12_20	YF_12_21	YF_12_22	YF_12_23	YF_12_24	YF_12_25	YF_12_26	YF_12_27	YF_12_28	YF_12_29	YF_12_30
Abundance of M	Pentacosene	316096	223808	299584	319488	320384	322816	317632	274944	319744	322176	318336	323904	188736	312128	331840
	Heptacosene	204864	78064	131048	187520	153792	169152	159552	97224	155776	219392	142976	185856	69456	132800	169728
Abundance of M+1	Pentacosene	104072	77752	102224	107888	106360	108456	109544	104152	105736	102248	111296	113080	63328	108120	109832
	Heptacosene	87864	33384	57368	77872	64256	70072	66288	48200	65488	91568	62160	80144	29120	54592	71920
Abundance of M+2	Pentacosene	21848	16528	21984	23784	23696	24224	22936	28592	22872	21304	23360	25016	14894	21656	22552
	Heptacosene	25536	9321	16528	23104	18648	20840	18464	20440	19144	25656	17984	22416	2314	16322	20688
Percentage of M+1	Pentacosene	32.9	34.7	34.1	33.8	33.2	33.6	34.5	37.9	33.1	31.7	35.0	34.9	33.6	34.6	33.1
	Heptacosene	42.9	42.8	43.8	41.5	41.8	41.4	41.5	49.6	42.0	41.7	43.5	43.1	41.9	41.1	42.4
Percentage of M+2	Pentacosene	6.9	7.4	7.3	7.4	7.4	7.5	7.2	10.4	7.2	6.6	7.3	7.7	7.9	6.9	6.8
	Heptacosene	12.5	11.9	12.6	12.3	12.1	12.3	11.6	21.0	12.3	11.7	12.6	12.1	3.3	12.3	12.2
Average M+n pentacosene and heptacosene values		23.8	24.2	24.5	23.8	23.6	23.7	23.7	29.7	23.6	22.9	24.6	24.5	21.7	23.7	23.6

0
0-20
20-40
40-60
60-80
80-100
100+
24.1%

Figure E.4: Data sheet showing page two, repeat two of the basic yellow data for *Formica lemani* at a dye concentration of 1 g/L.

Basic yellow 1g / L Repeat 3		*Formica lemani*														
		YF_13_1	YF_13_2	YF_13_3	YF_13_4	YF_13_5	YF_13_6	YF_13_7	YF_13_8	YF_13_9	YF_13_10	YF_13_11	YF_13_12	YF_13_13	YF_13_14	YF_13_15
Abundance of M	Pentacosene	299008	313920	324352	306304	321152	318400	324864	325824	291584	318464	315520	313664	336512	333952	329536
	Heptacosene	98760	134976	194304	127368	139136	216896	153088	156928	126000	124768	158144	220736	156736	154496	180800
Abundance of M+1	Pentacosene	107368	115000	117496	112656	115288	113960	119416	115376	111504	113960	119016	117888	122272	121456	120056
	Heptacosene	46640	65144	93800	61288	66280	100520	74048	75560	65504	59744	82080	112280	74752	74472	86056
Abundance of M+2	Pentacosene	25976	28320	29584	30320	27280	26992	27768	28400	30672	29896	32288	31600	30176	29128	31288
	Heptacosene	15698	21568	31776	21080	22608	33920	24768	24712	23768	20888	31048	38192	26216	25256	28736
Percentage of M+1	Pentacosene	35.9	36.6	36.2	36.8	35.9	35.8	36.8	35.4	38.2	35.8	37.7	37.6	36.3	36.4	36.4
	Heptacosene	47.2	48.3	48.3	48.1	47.6	46.3	48.4	48.1	52.0	47.9	51.9	50.9	47.7	48.2	47.6
Percentage of M+2	Pentacosene	8.7	9.0	9.1	9.9	8.5	8.5	8.5	8.7	10.5	9.4	10.2	10.1	9.0	8.7	9.5
	Heptacosene	15.9	16.0	16.4	16.6	16.2	15.6	16.2	15.7	18.9	16.7	19.6	17.3	16.7	16.3	15.9
Average M+n pentacosene and heptacosene values		26.9	27.5	27.5	27.8	27.1	26.6	27.5	27.0	29.9	27.4	29.9	29.0	27.4	27.4	27.4

0
0-20
20-40
40-60
60-80
80-100
100+

Figure E.5: Data sheet showing page one, repeat three of the basic yellow data for *Formica lemani* at a dye concentration of 1 g/L.

Basic yellow 1g / L Repeat 3		*Formica lemani*													
		YF_13_16	YF_13_17	YF_13_18	YF_13_19	YF_13_20	YF_13_21	YF_13_22	YF_13_23	YF_13_24	YF_13_25	YF_13_26	YF_13_27	YF_13_28	YF_13_29
Abundance of M	Pentacosene	292160	226880	328000	300992	296960	334208	264192	339648	228480	326592	337088	257024	214336	339712
	Heptacosene	103696	57480	167168	132736	116856	192192	87296	180992	75272	111288	147968	91752	63592	136768
Abundance of M+1	Pentacosene	112232	87864	125024	109936	117064	120376	100056	124512	87736	114968	124616	91624	77792	123888
	Heptacosene	51904	31888	83504	64640	59784	92312	45312	85896	37752	54240	71416	40672	30216	65304
Abundance of M+2	Pentacosene	31592	26784	35752	27160	30808	28648	28272	29792	24728	28816	31576	23152	19680	30448
	Heptacosene	19032	13595	30216	21920	21320	30920	16960	27888	14305	18880	25176	14159	10477	22304
Percentage of M+1	Pentacosene	38.4	38.7	38.1	36.5	39.4	36.0	37.9	36.7	38.4	35.2	37.0	35.6	36.3	36.5
	Heptacosene	50.1	55.5	50.0	48.7	51.2	48.0	51.9	47.5	50.2	48.7	48.3	44.3	47.5	47.7
Percentage of M+2	Pentacosene	10.8	11.8	10.9	9.0	10.4	8.6	10.7	8.8	10.8	8.8	9.4	9.0	9.2	9.0
	Heptacosene	18.4	23.7	18.1	16.5	18.2	16.1	19.4	15.4	19.0	17.0	17.0	15.4	16.5	16.3
Average M+n pentacosene and heptacosene values		29.4	32.4	29.3	27.7	29.8	27.2	30.0	27.1	29.6	27.4	27.9	26.1	27.4	27.4

0
0-20
20-40
40-60
60-80
80-100
100+
28.1%

Figure E.6: Data sheet showing page two, repeat three of the basic yellow data for *Formica lemani* at a dye concentration of 1 g/L.

Basic yellow 2g / L Repeat 1		*Formica lemani*														
		YF_21_1	YF_21_2	YF_21_3	YF_21_4	YF_21_5	YF_21_6	YF_21_7	YF_21_8	YF_21_9	YF_21_10	YF_21_11	YF_21_12	YF_21_13	YF_21_14	YF_21_15
Abundance of M	Pentacosene	344256	305728	263552	357440	366464	366464	380032	391424	266880	377664	106928		108912	118872	186496
	Heptacosene	109560	83544	85152	112416	137408	157568	150592	196480	65688	212992	21536		25024	27920	48504
Abundance of M+1	Pentacosene	105840	94296	83968	105112	116088	107808	114128	117136	83216	116960	34704		32256	37192	60232
	Heptacosene	41088	31456	31576	41112	54136	56424	53840	70416	24304	76352	8770		9091	10944	18720
Abundance of M+2	Pentacosene	19112	16255	15128	19032	24176	20104	21016	20168	14593	22128	6873		6196	6492	11408
	Heptacosene	10328	7350	7975	10127	14715	13548	12704	16178	5724	18432	2645		2467	2351	5446
Percentage of M+1	Pentacosene	30.7	30.8	31.9	29.4	31.7	29.4	30.0	29.9	31.2	31.0	32.5		29.6	31.3	32.3
	Heptacosene	37.5	37.7	37.1	36.6	39.4	35.8	35.8	35.8	37.0	35.8	40.7		36.3	39.2	38.6
Percentage of M+2	Pentacosene	5.6	5.3	5.7	5.3	6.6	5.5	5.5	5.2	5.5	5.9	6.4		5.7	5.5	6.1
	Heptacosene	9.4	8.8	9.4	9.0	10.7	8.6	8.4	8.2	8.7	8.7	12.3		9.9	8.4	11.2
Average M+n pentacosene and heptacosene values		20.8	20.7	21.0	20.1	22.1	19.8	19.9	19.8	20.6	20.3	23.0		20.4	21.1	22.1

0
0-20
20-40
40-60
60-80
80-100
100+

Figure E.7: Data sheet showing page one, repeat one of the basic yellow data for *Formica lemani* at a dye concentration of 2 g/L.

Basic yellow 2g / L Repeat 1		*Formica lemani*											
		YF_21_16	YF_21_17	YF_21_18	YF_21_19	YF_21_20	YF_21_21	YF_21_22	YF_21_23	YF_21_24	YF_21_25	YF_21_26	YF_21_27
Abundance of M	Pentacosene	121080	209152	201600	117296	203200	224448	171840	151232	200448	136128	171904	275328
	Heptacosene	32584	65584	75552	35848	62768	73960	52856	43096	59216	41304	47984	91936
Abundance of M+1	Pentacosene	36560	65528	61088	36776	62544	68968	54136	46280	61336	42288	52392	82024
	Heptacosene	12764	24352	28168	13405	23584	27024	20336	16568	20744	15326	19032	32304
Abundance of M+2	Pentacosene	6947	11565	11206	6491	11486	12874	9916	8490	10532	7075	9751	14445
	Heptacosene	2824	5582	6740	3147	5903	6119	4897	3931	5368	3797	4570	7950
Percentage of M+1	Pentacosene	30.2	31.3	30.3	31.4	30.8	30.7	31.5	30.6	30.6	31.1	30.5	29.8
	Heptacosene	39.2	37.1	37.3	37.4	37.6	36.5	38.5	38.4	35.0	37.1	39.7	35.1
Percentage of M+2	Pentacosene	5.7	5.5	5.6	5.5	5.7	5.7	5.8	5.6	5.3	5.2	5.7	5.2
	Heptacosene	8.7	8.5	8.9	8.8	9.4	8.3	9.3	9.1	9.1	9.2	9.5	8.6
Average M+n pentacosene and heptacosene values		20.9	20.6	20.5	20.8	20.9	20.3	21.3	20.9	20.0	20.6	21.3	19.7

0
0-20
20-40
40-60
60-80
80-100
100+
20.8%

Figure E.8: Data sheet showing page two, repeat one of the basic yellow data for *Formica lemani* at a dye concentration of 2 g/L.

Basic yellow 2g / L Repeat 2		*Formica lemani*														
		YF_22_1	YF_22_2	YF_22_3	YF_22_4	YF_22_5	YF_22_6	YF_22_7	YF_22_8	YF_22_9	YF_22_10	YF_22_11	YF_22_12	YF_22_13	YF_22_14	YF_22_15
Abundance of M	Pentacosene	55544	41856	54888	170944	99976	96864	154816	151680	25456	125800	16544	21776	222464	106088	72184
	Heptacosene	12612	9049	11590	57240	24448	29376	38536	39368	6790	30952	4646	6093	85736	36336	22056
Abundance of M+1	Pentacosene	17488	13827	18696	57208	32304	31752	50264	49192	7951	42072	5928	7240	71256	36352	24608
	Heptacosene	5697	3580	4989	22768	9661	10960	15517	15089	2998	13680	1722	2777	32600	15266	9245
Abundance of M+2	Pentacosene	3538	2505	3900	11159	6772	6094	9500	9929	1711	8632	1079	1550	14690	8029	5463
	Heptacosene	1720	1117	1240	6500	2998	3484	4043	3831	732	4092	694	841	9320	4592	2518
Percentage of M+1	Pentacosene	31.5	33.0	34.1	33.5	32.3	32.8	32.5	32.4	31.2	33.4	35.8	33.2	32.0	34.3	34.1
	Heptacosene	45.2	39.6	43.0	39.8	39.5	37.3	40.3	38.3	44.2	44.2	37.1	45.6	38.0	42.0	41.9
Percentage of M+2	Pentacosene	6.4	6.0	7.1	6.5	6.8	6.3	6.1	6.5	6.7	6.9	6.5	7.1	6.6	7.6	7.6
	Heptacosene	13.6	12.3	10.7	11.4	12.3	11.9	10.5	9.7	10.8	13.2	14.9	13.8	10.9	12.6	11.4
Average M+n pentacosene and heptacosene values		24.2	22.7	23.7	22.8	22.7	22.1	22.3	21.8	23.2	24.4	23.6	24.9	21.9	24.1	23.7

0
0-20
20-40
40-60
60-80
80-100
100+

Figure E.9: Data sheet showing page one, repeat two of the basic yellow data for *Formica lemani* at a dye concentration of 2 g/L.

Basic yellow 2g / L Repeat 2		*Formica lemani*												
		YF_22_16	YF_22_17	YF_22_18	YF_22_19	YF_22_20	YF_22_21	YF_22_22	YF_22_23	YF_22_24	YF_22_25	YF_22_26	YF_22_27	YF_22_28
Abundance of M	Pentacosene	19776	41360	95888	166400	272256	153472	79632	58440	50312	104032	91192	127488	148736
	Heptacosene	6859	10735	27192	46264	88680	42136	19520	12779	11625	24232	31032	36688	32272
Abundance of M+1	Pentacosene	6259	13814	31520	56032	86088	50200	24896	19616	16744	37448	28200	40424	49296
	Heptacosene	2623	4570	10927	19072	35056	17456	7870	5688	5221	10866	12634	15395	13095
Abundance of M+2	Pentacosene	1237	2895	6925	10693	17720	9684	4961	4616	3639	8620	5701	8274	10292
	Heptacosene	870	1294	3442	5780	10911	4905	2313	1693	1685	3255	3533	4327	3916
Percentage of M+1	Pentacosene	31.6	33.4	32.9	33.7	31.6	32.7	31.3	33.6	33.3	36.0	30.9	31.7	33.1
	Heptacosene	38.2	42.6	40.2	41.2	39.5	41.4	40.3	44.5	44.9	44.8	40.7	42.0	40.6
Percentage of M+2	Pentacosene	6.3	7.0	7.2	6.4	6.5	6.3	6.2	7.9	7.2	8.3	6.3	6.5	6.9
	Heptacosene	12.7	12.1	12.7	12.5	12.3	11.6	11.8	13.2	14.5	13.4	11.4	11.8	12.1
Average M+n pentacosene and heptacosene values		22.2	23.8	23.2	23.5	22.5	23.0	22.4	24.8	25.0	25.6	22.3	23.0	23.2

0
0-20
20-40
40-60
60-80
80-100
100+

23.3%

Figure E.10: Data sheet showing page two, repeat two of the basic yellow data for *Formica lemani* at a dye concentration of 2 g/L.

Basic yellow 2g / L Repeat 3		*Formica lemani*														
		YF_23_1	YF_23_2	YF_23_3	YF_23_4	YF_23_5	YF_23_6	YF_23_7	YF_23_8	YF_23_9	YF_23_10	YF_23_11	YF_23_12	YF_23_13	YF_23_14	YF_23_15
Abundance of M	Pentacosene	166592	98808	123104	122928	159360	266624	177472	24248	128544	254400	52296	161472	192384	164800	310208
	Heptacosene	43936	26328	34504	41384	50096	79000	54584	7422	39192	82728	14060	43360	59408	60600	141065
Abundance of M+1	Pentacosene	53080	31616	40200	39960	49640	84160	58360	7728	41680	80296	16768	52200	57488	55304	97504
	Heptacosene	16976	10470	14782	16297	19712	31080	22112	2972	16544	33064	5871	17544	22080	25672	54824
Abundance of M+2	Pentacosene	11046	6250	7952	9302	9996	18440	12630	1716	7878	15726	3803	11333	12180	13270	19736
	Heptacosene	4513	3052	4207	5217	5348	8618	6451	1081	4838	8993	1838	5167	6454	7387	15071
Percentage of M+1	Pentacosene	31.9	32.0	32.7	32.5	31.1	31.6	32.9	31.9	32.4	31.6	32.1	32.3	29.9	33.6	31.4
	Heptacosene	38.6	39.8	42.8	39.4	39.3	39.3	40.5	40.0	42.2	40.0	41.8	40.5	37.2	42.4	38.9
Percentage of M+2	Pentacosene	6.6	6.3	6.5	7.6	6.3	6.9	7.1	7.1	6.1	6.2	7.3	7.0	6.3	8.1	6.4
	Heptacosene	10.3	11.6	12.2	12.6	10.7	10.9	11.8	14.6	12.3	10.9	13.1	11.9	10.9	12.2	10.7
Average M+n pentacosene and heptacosene values		21.9	22.4	23.5	23.0	21.9	22.2	23.1	23.4	23.3	22.1	23.5	22.9	21.1	24.0	21.8

0
0-20
20-40
40-60
60-80
80-100
100+

Figure E.11: Data sheet showing page one, repeat three of the basic yellow data for *Formica lemani* at a dye concentration of 2 g/L.

Basic yellow 2g / L Repeat 3		*Formica lemani*														
		YF_23_16	YF_23_17	YF_23_18	YF_23_19	YF_23_20	YF_23_21	YF_23_22	YF_23_23	YF_23_24	YF_23_25	YF_23_26	YF_23_27	YF_23_28	YF_23_29	YF_23_30
Abundance of M	Pentacosene	212544	72728	104608	118000	130696	193344	173248	64880	160128	216768	204800	227520	302976	244032	142592
	Heptacosene	71224	17064	26712	32512	29640	56640	50320	17928	46376	67936	69688	70336	110584	78760	45016
Abundance of M+1	Pentacosene	68256	23640	34152	37712	41920	63024	56968	22072	51648	70392	67264	74352	97864	80592	45328
	Heptacosene	28968	7341	10542	14060	12466	22480	21504	7692	17840	28352	27504	28312	45128	31008	18528
Abundance of M+2	Pentacosene	13767	5862	6662	8032	9274	12995	12022	4312	10282	14172	13262	15358	21288	16680	9830
	Heptacosene	8527	2402	3065	4059	3395	7022	6813	2603	5853	8446	7942	8130	13368	9395	5739
Percentage of M+1	Pentacosene	32.1	32.5	32.6	32.0	32.1	32.6	32.9	34.0	32.3	32.5	32.8	32.7	32.3	33.0	31.8
	Heptacosene	40.7	43.0	39.5	43.2	42.1	39.7	42.7	42.9	38.5	41.7	39.5	40.3	40.8	39.4	41.2
Percentage of M+2	Pentacosene	6.5	8.1	6.4	6.8	7.1	6.7	6.9	6.6	6.4	6.5	6.5	6.8	7.0	6.8	6.9
	Heptacosene	12.0	14.1	11.5	12.5	11.5	12.4	13.5	14.5	12.6	12.4	11.4	11.6	12.1	11.9	12.7
Average M+n pentacosene and heptacosene values		22.8	24.4	22.5	23.6	23.2	22.9	24.0	24.5	22.4	23.3	22.5	22.8	23.1	22.8	23.1

0
0-20
20-40
40-60
60-80
80-100
100+
22.9%

Figure E.12: Data sheet showing page two, repeat three of the basic yellow data for *Formica lemani* at a dye concentration of 2 g/L.

Basic yellow 5g / L Repeat 1		*Formica lemani*														
		YF_51_1	YF_51_2	YF_51_3	YF_51_4	YF_51_5	YF_51_6	YF_51_7	YF_51_8	YF_51_9	YF_51_10	YF_51_11	YF_51_12	YF_51_13	YF_51_14	YF_51_15
Abundance of M	Pentacosene	39192	64016	225920	183616	102976	67248	139072	188544	198464	83632	93944	219200	142720	109512	142528
	Heptacosene	7864	17648	70032	52072	21864	18352	38136	50080	56768	22248	19416	68248	33616	25680	41344
Abundance of M+1	Pentacosene	12073	19800	70224	52480	29544	20840	44056	56856	60192	26400	28200	64784	43576	34776	44512
	Heptacosene	2727	6137	25632	20008	8309	7076	13189	18472	19184	7482	7523	24544	12791	9353	15369
Abundance of M+2	Pentacosene	1803	3429	11504	9532	5161	3508	6798	9005	10033	4735	4553	11947	7525	6250	7395
	Heptacosene	579	1481	6389	4374	1895	1700	3055	4300	4401	1860	1692	5706	3595	2004	3892
Percentage of M+1	Pentacosene	30.8	30.9	31.1	28.6	28.7	31.0	31.7	30.2	30.3	31.6	30.0	29.6	30.5	31.8	31.2
	Heptacosene	34.7	34.8	36.6	38.4	38.0	38.6	34.6	36.9	33.8	33.6	38.7	36.0	38.1	36.4	37.2
Percentage of M+2	Pentacosene	4.6	5.4	5.1	5.2	5.0	5.2	4.9	4.8	5.1	5.7	4.8	5.5	5.3	5.7	5.2
	Heptacosene	7.4	8.4	9.1	8.4	8.7	9.3	8.0	8.6	7.8	8.4	8.7	8.4	10.7	7.8	9.4
Average M+n pentacosene and heptacosene values		19.4	19.9	20.5	20.1	20.1	21.0	19.8	20.1	19.2	19.8	20.6	19.8	21.1	20.4	20.8

0
0-20
20-40
40-60
60-80
80-100
100+

Figure E.13: Data sheet showing page one, repeat one of the basic yellow data for *Formica lemani* at a dye concentration of 5 g/L.

Basic yellow 5g / L Repeat 1		*Formica lemani*											
		YF_51_16	YF_51_17	YF_51_18	YF_51_19	YF_51_20	YF_51_21	YF_51_22	YF_51_23	YF_51_24	YF_51_25	YF_51_26	YF_51_27
Abundance of M	Pentacosene	47592	118112	143488	62872	146432	92976	14960	60568	63464	38840	132736	242560
	Heptacosene	10635	28776	34888	15757	34136	21680	3389	10791	13502	8523	35672	70008
Abundance of M+1	Pentacosene	14608	35896	44688	20176	44176	29136	4797	19464	19120	11707	38280	72112
	Heptacosene	3347	10293	13045	6324	13030	7621	1465	3972	4596	3355	12722	26224
Abundance of M+2	Pentacosene	2285	5503	8199	3250	7960	4298	1020	3471	3168	2221	7282	12728
	Heptacosene	891	2420	3525	1326	2797	1911	353	786	929	880	2557	5751
Percentage of M+1	Pentacosene	30.7	30.4	31.1	32.1	30.2	31.3	32.1	32.1	30.1	30.1	28.8	29.7
	Heptacosene	31.5	35.8	37.4	40.1	38.2	35.2	43.2	36.8	34.0	39.4	35.7	37.5
Percentage of M+2	Pentacosene	4.8	4.7	5.7	5.2	5.4	4.6	6.8	5.7	5.0	5.7	5.5	5.2
	Heptacosene	8.4	8.4	10.1	8.4	8.2	8.8	10.4	7.3	6.9	10.3	7.2	8.2
Average M+n pentacosene and heptacosene values		18.8	19.8	21.1	21.5	20.5	20.0	23.1	20.5	19.0	21.4	19.3	20.2

0
0-20
20-40
40-60
60-80
80-100
100+
20.3%

Figure E.14: Data sheet showing page two, repeat one of the basic yellow data for *Formica lemani* at a dye concentration of 5 g/L.

Basic yellow 5g / L Repeat 3		*Formica lemani*														
		YF_53_1	YF_53_2	YF_53_3	YF_53_4	YF_53_5	YF_53_6	YF_53_7	YF_53_8	YF_53_9	YF_53_10	YF_53_11	YF_53_12	YF_53_13	YF_53_14	YF_53_15
Abundance of M	Pentacosene	356288	367680	354304		371904	273664	313536	319296	222144	242624	372416	351424	387968	357760	345408
	Heptacosene	231040	138496	264192		145152	74568	111624	95256	93608	75544	264256	144384	154304	140864	109072
Abundance of M+1	Pentacosene	118832	122456	115272		123304	87840	110160	104864	75376	80888	120712	117864	123472	117480	111872
	Heptacosene	97216	60256	108144		59528	31736	50056	38728	37288	33264	109232	59392	65048	57616	43440
Abundance of M+2	Pentacosene	26064	28256	23184		26000	17323	25392	22392	15134	19264	23544	23344	24704	23952	22232
	Heptacosene	29736	19384	31512		17184	9329	16744	11142	11299	11125	30816	17240	18280	16872	12434
Percentage of M+1	Pentacosene	33.4	33.3	32.5		33.2	32.1	35.1	32.8	33.9	33.3	32.4	33.5	31.8	32.8	32.4
	Heptacosene	42.1	43.5	40.9		41.0	42.6	44.8	40.7	39.8	44.0	41.3	41.1	42.2	40.9	39.8
Percentage of M+2	Pentacosene	7.3	7.7	6.5		7.0	6.3	8.1	7.0	6.8	7.9	6.3	6.6	6.4	6.7	6.4
	Heptacosene	12.9	14.0	11.9		11.8	12.5	15.0	11.7	12.1	14.7	11.7	11.9	11.8	12.0	11.4
Average M+n pentacosene and heptacosene values		23.9	24.6	23.0		23.2	23.4	25.8	23.1	23.2	25.0	22.9	23.3	23.0	23.1	22.5

0
0-20
20-40
40-60
60-80
80-100
100+

Figure E.15: Data sheet showing page one, repeat three of the basic yellow data for *Formica lemani* at a dye concentration of 5 g/L.

Basic yellow 5g / L Repeat 3		*Formica lemani*														
		YF_53_16	YF_53_17	YF_53_18	YF_53_19	YF_53_20	YF_53_21	YF_53_22	YF_53_23	YF_53_24	YF_53_25	YF_53_26	YF_53_27	YF_53_28	YF_53_29	YF_53_30
Abundance of M	Pentacosene	383936	392000	395456	388224	243520	355456	379136	391680	388416	395712	398400		306560	239360	141312
	Heptacosene	135616	219008	167040	178368	67088	132608	154496	201408	172736	159232	184128		86512	70904	33536
Abundance of M+1	Pentacosene	127104	128040	123168	124896	78024	113656	124408	126160	129808	133120	125992		104440	79312	46752
	Heptacosene	56176	89008	67728	76792	28496	51872	63856	79336	75424	63672	74240		34552	31984	14905
Abundance of M+2	Pentacosene	28008	26976	234968	27048	15673	22536	25832	27064	30560	26280	26016		20392	15872	10355
	Heptacosene	15895	25656	19896	23272	7862	15916	18840	22648	23144	19024	21888		11017	8547	4389
Percentage of M+1	Pentacosene	33.1	32.7	31.1	32.2	32.0	32.0	32.8	32.2	33.4	33.6	31.6		34.1	33.1	33.1
	Heptacosene	41.4	40.6	40.5	43.1	42.5	39.1	41.3	39.4	43.7	40.0	40.3		39.9	45.1	44.4
Percentage of M+2	Pentacosene	7.3	6.9	59.4	7.0	6.4	6.3	6.8	6.9	7.9	6.6	6.5		6.7	6.6	7.3
	Heptacosene	11.7	11.7	11.9	13.0	11.7	12.0	12.2	11.2	13.4	11.9	11.9		12.7	12.1	13.1
Average M+n pentacosene and heptacosene values		23.4	23.0	35.8	23.8	23.2	22.4	23.3	22.4	24.6	23.1	22.6		23.3	24.2	24.5

0
0-20
20-40
40-60
60-80
80-100
100+

23.9%

Figure E.16: Data sheet showing page two, repeat three of the basic yellow data for *Formica lemani* at a dye concentration of 5 g/L.

Basic yellow Dye only		*Formica lemani*														
		YF_DY_1	YF_DY_2	YF_DY_3	YF_DY_4	YF_DY_5	YF_DY_6	YF_DY_7	YF_DY_8	YF_DY_9	YF_DY_10	YF_DY_11	YF_DY_12	YF_DY_13	YF_DY_14	YF_DY_15
Abundance of M	Pentacosene	346368	336384	287616	359936	161472	309888	346688	167808	235520	360448	228352	151744	343232	260288	230336
	Heptacosene	161088	196032	156928	229824	51808	134528	167424	51328	103488	264896	99432	56656	187456	124856	93304
Abundance of M+1	Pentacosene	92064	91056	76592	94272	43264	85712	95368	45072	62560	97928	61376	41448	93912	70128	61480
	Heptacosene	47008	54392	44704	67176	14881	40296	48456	14844	30184	76232	29456	16952	53600	36064	27096
Abundance of M+2	Pentacosene	11803	10532	9686	12073	6121	11477	11708	5305	7653	12276	7607	5774	12144	8905	8281
	Heptacosene	6043	7612	6217	10044	2434	5752	6682	2103	4296	10581	4309	2466	7724	5371	4184
Percentage of M+1	Pentacosene	26.6	27.1	26.6	26.2	26.8	27.7	27.5	26.9	26.6	27.2	26.9	27.3	27.4	26.9	26.7
	Heptacosene	29.2	27.7	28.5	29.2	28.7	30.0	28.9	28.9	29.2	28.8	29.6	29.9	28.6	28.9	29.0
Percentage of M+2	Pentacosene	3.4	3.1	3.4	3.4	3.8	3.7	3.4	3.2	3.2	3.4	3.3	3.8	3.5	3.4	3.6
	Heptacosene	3.8	3.9	4.0	4.4	4.7	4.3	4.0	4.1	4.2	4.0	4.3	4.4	4.1	4.3	4.5
Average M+n pentacosene and heptacosene values		15.7	15.5	15.6	15.8	16.0	16.4	16.0	15.8	15.8	15.8	16.0	16.3	15.9	15.9	16.0

0
0-20
20-40
40-60
60-80
80-100
100+

Figure E.17: Data sheet showing page one of the raw data for the basic yellow, dye only control for *Formica lemani*

Basic yellow Dye only		*Formica lemani*						
		YF_DY_16	YF_DY_17	YF_DY_18	YF_DY_19	YF_DY_20	YF_DY_21	YF_DY_22
Abundance of M	Pentacosene	300992	375552	290752		296640	378496	335936
	Heptacosene	108232	265280	138944		115496	237888	189376
Abundance of M+1	Pentacosene	77680	100848	75224		79200	100592	86712
	Heptacosene	31376	74800	40976		34240	67288	55880
Abundance of M+2	Pentacosene	9575	12430	9545		10396	12165	11563
	Heptacosene	4382	10203	5426		4787	9586	7823
Percentage of M+1	Pentacosene	25.8	26.9	25.9		26.7	26.6	25.8
	Heptacosene	29.0	28.2	29.5		29.6	28.3	29.5
Percentage of M+2	Pentacosene	3.2	3.3	3.3		3.5	3.2	3.4
	Heptacosene	4.0	3.8	3.9		4.1	4.0	4.1
Average M+n pentacosene and heptacosene values		15.5	15.6	15.6		16.0	15.5	15.7

0
0-20
20-40
40-60
60-80
80-100
100+

15.8%

Figure E.18: Data sheet showing page two of the raw data for the basic yellow, dye only control for *Formica lemani*

Sodium [$^{13}C_2$]acetate only		*Formica lemani*																
		FSA_1	FSA_2	FSA_3	FSA_4	FSA_5	FSA_6	FSA_7	FSA_8	FSA_9	FSA_10	FSA_11	FSA_12	FSA_13	FSA_14	FSA_15	FSA_16	FSA_17
Abundance of M	Pentacosene	232192	231360	327232	342592	231872	260032	296064	178816	288832	223872	149760	168512	237056	155584	105632	150080	177792
	Heptacosene	76784	81088	147584	128256	49784	99432	50144	72152	143680	96312	44872	72464	82744	75696	32848	10022	61384
Abundance of M+1	Pentacosene	92760	93368	136000	137088	95128	105048	119056	74168	110888	90400	62816	65808	98048	62576	44384	59224	72328
	Heptacosene	43504	44336	81696	71016	30960	55712	23472	41520	77920	54440	25368	38504	48144	40680	18696	4503	35784
Abundance of M+2	Pentacosene	30712	33072	43240	39760	33400	33304	37632	25096	33384	29720	21968	20256	31736	19216	14678	19136	24168
	Heptacosene	20360	20432	35776	31488	14025	25552	10477	18104	31312	24640	11547	16057	21464	17376	8872	1960	15337
Percentage of M+1	Pentacosene	39.9	40.4	41.6	40.0	41.0	40.4	40.2	41.5	38.4	40.4	41.9	39.1	41.4	40.2	42.0	39.5	40.7
	Heptacosene	56.7	54.7	55.4	55.4	62.2	56.0	46.8	57.5	54.2	56.5	56.5	53.1	58.2	53.7	56.9	44.9	58.3
Percentage of M+2	Pentacosene	13.2	14.3	13.2	11.6	14.4	12.8	12.7	14.0	11.6	13.3	14.7	12.0	13.4	12.4	13.9	12.8	13.6
	Heptacosene	26.5	25.2	24.2	24.6	28.2	25.7	20.9	25.1	21.8	25.6	25.7	22.2	25.9	23.0	27.0	19.6	25.0
Average M+n pentacosene and heptacosene values		34.1	33.6	33.6	32.9	36.4	33.7	30.2	34.5	31.5	33.9	34.7	31.6	34.7	32.3	35.0	29.2	34.4

0
0-20
20-40
40-60
60-80
80-100
100+

Figure E.19: Data sheet showing the raw data for the sodium [$^{13}C_2$]acetate control for *Formica lemani*

Basic yellow 1g / L Repeat 1		*Myrmica rubra*			
		YR_11_1	YR_11_2	YR_11_3	YR_11_4
Abundance of M	Pentacosane	10956	27872	16220	6761
	Heptacosane	3178	9267	5091	2442
Abundance of M+1	Pentacosane	3960	9911	5939	1970
	Heptacosane	1147	3604	1881	1024
Abundance of M+2	Pentacosane	769	2684	1707	419
	Heptacosane	369	1009	777	239
Percentage of M+1	Pentacosane	36.1	35.6	36.6	29.1
	Heptacosane	36.1	38.9	36.9	41.9
Percentage of M+2	Pentacosane	7.0	9.6	10.5	6.2
	Heptacosane	11.6	10.9	15.3	9.8
Average M+n pentacosane and heptacosane values		22.7	23.7	24.8	21.8

0
0-20
20-40
40-60
60-80
80-100
100+

23.3%

Figure E.20: Data sheet showing repeat one of the basic yellow data for *Myrmica rubra* at a dye concentration of 1 g/L.

Basic yellow 1g / L Repeat 2		*Myrmica rubra*									
		YM_12_1	YM_12_2	YM_12_3	YM_12_4	YM_12_5	YM_12_6	YM_12_7	YM_12_8	YM_12_9	YM_12_10
Abundance of M	Pentacosane	18008	14282	16800	14153	17320	27880	15702	21912	45944	20744
	Heptacosane	6256	5044	6468	4711	5417	7698	5622	6278	18208	3725
Abundance of M+1	Pentacosane	5220	7570	6180	4419	6239	11299	6148	11567	16019	8051
	Heptacosane	2230	1930	2980	2002	1891	2779	1980	2541	6470	2023
Abundance of M+2	Pentacosane	1449	2209	1578	1216	1760	3209	1340	4558	4588	3704
	Heptacosane	453	1160	804	449	830	647	642	1007	1956	862
Percentage of M+1	Pentacosane	29.0	53.0	36.8	31.2	36.0	40.5	39.2	52.8	34.9	38.8
	Heptacosane	35.6	38.3	46.1	42.5	34.9	36.1	35.2	40.5	35.5	54.3
Percentage of M+2	Pentacosane	8.0	15.5	9.4	8.6	10.2	11.5	8.5	20.8	10.0	17.9
	Heptacosane	7.2	23.0	12.4	9.5	15.3	8.4	11.4	16.0	10.7	23.1
Average M+n pentacosane and heptacosane values		20.0	32.4	26.2	23.0	24.1	24.1	23.6	32.5	22.8	33.5

0
0-20
20-40
40-60
60-80
80-100
100+
26.2%

Figure E.21: Data sheet showing repeat two of the basic yellow data for *Myrmica rubra* at a dye concentration of 1 g/L.

Basic yellow 2g / L Repeat 1		*Myrmica rubra*							
		YM_21_1	YM_21_2	YM_21_3	YM_21_4	YM_21_5	YM_21_6	YM_21_7	YM_21_8
Abundance of M	Pentacosane	20744	25960	48112	24264	35200	29344	75648	16872
	Heptacosane	3725	11598	10812	6496	8855	6180	26968	5320
Abundance of M+1	Pentacosane	8051	10582	17976	9695	13293	12685	30016	9460
	Heptacosane	2023	4953	3959	2840	4445	2259	10630	2721
Abundance of M+2	Pentacosane	3704	3983	5343	2907	3288	5070	8437	4324
	Heptacosane	862	1988	1455	1080	1400	921	3066	1599
Percentage of M+1	Pentacosane	38.8	40.8	37.4	40.0	37.8	43.2	39.7	56.1
	Heptacosane	54.3	42.7	36.6	43.7	50.2	36.6	39.4	51.1
Percentage of M+2	Pentacosane	17.9	15.3	11.1	12.0	9.3	17.3	11.2	25.6
	Heptacosane	23.1	17.1	13.5	16.6	15.8	14.9	11.4	30.1
Average M+n pentacosane and heptacosane values		33.5	29.0	24.6	28.1	28.3	28.0	25.4	40.7

0
0-20
20-40
40-60
60-80
80-100
100+
29.7%

Figure E.22: Data sheet showing repeat one of the basic yellow data for *Myrmica rubra* at a dye concentration of 2 g/L.

Basic yellow 2g / L Repeat 2		*Myrmica rubra*																
		YM_22_1	YM_22_2	YM_22_3	YM_22_4	YM_22_5	YM_22_6	YM_22_7	YM_22_8	YM_22_9	YM_22_10	YM_22_11	YM_22_12	YM_22_13	YM_22_14	YM_22_15	YM_22_16	YM_22_17
Abundance of M	Pentacosane	11650	22520	21976	27616	35248	19472	14291	12109	23744	39616	25024	10058	23096	25704	12952	24696	16640
	Heptacosane	4553	5707	8660	10237	11440	8143	6282	6658	9649	14921	9800	4455	6592	10385	4241	8433	7153
Abundance of M+1	Pentacosane	5948	12134	8208	12469	13325	8488	5917	3859	13155	19512	12788	4838	11987	12693	6655	12138	5411
	Heptacosane	1435	3340	4120	4888	3744	3263	1965	1896	4007	6786	5115	1780	2771	4017	1748	3090	2741
Abundance of M+2	Pentacosane	1611	4739	2606	3958	4340	4375	2148	1031	4785	7790	5267	1974	4898	5271	2303	4114	1888
	Heptacosane	1053	1083	1138	1419	1763	1476	957	518	2616	3118	1820	553	1562	1665	853	1205	518
Percentage of M+1	Pentacosane	51.1	53.9	37.3	45.2	37.8	43.6	41.4	31.9	55.4	49.3	51.1	48.1	51.9	49.4	51.4	49.1	32.5
	Heptacosane	31.5	58.5	47.6	47.7	32.7	40.1	31.3	28.5	41.5	45.5	52.2	40.0	42.0	38.7	41.2	36.6	38.3
Percentage of M+2	Pentacosane	13.8	21.0	11.9	14.3	12.3	22.5	15.0	8.5	20.2	19.7	21.0	19.6	21.2	20.5	17.8	16.7	11.3
	Heptacosane	23.1	19.0	13.1	13.9	15.4	18.1	15.2	7.8	27.1	20.9	18.6	12.4	23.7	16.0	20.1	14.3	7.2
Average M+n pentacosane and heptacosane values		29.9	38.1	27.5	30.3	24.6	31.1	25.7	19.2	36.0	33.8	35.7	30.0	34.7	31.2	32.6	29.2	22.4

0	
0-20	
20-40	
40-60	
60-80	
80-100	
100+	
	31.1%

Figure E.23: Data sheet showing repeat two of the basic yellow data for *Myrmica rubra* at a dye concentration of 2 g/L.

Basic yellow 2g / L Repeat 3		*Myrmica rubra*														
		YM_23_1	YM_23_2	YM_23_3	YM_23_4	YM_23_5	YM_23_6	YM_23_7	YM_23_8	YM_23_9	YM_23_10	YM_23_11	YM_23_12	YM_23_13	YM_23_14	YM_23_15
Abundance of M	Pentacosane	43208	33032	23048	35576	42848	35200	51600	45168	58656	33032	22928	14917	14747	10497	23072
	Heptacosane	21528	13990	7289	16528	19856	15293	16576	16880	24320	13541	9116	5710	6228	3659	7470
Abundance of M+1	Pentacosane	18888	15034	11803	16392	15688	14570	20864	17576	24248	18936	8480	7571	6701	6069	10372
	Heptacosane	8216	5847	3667	7426	8154	6729	6616	6985	9077	6120	3071	2016	1727	1407	3352
Abundance of M+2	Pentacosane	6581	6871	6282	6016	4900	6404	7873	6756	7528	7826	3108	2483	2490	3029	4121
	Heptacosane	3139	2380	2174	2124	2193	2454	3098	2985	2888	2748	1250	734	1197	792	1663
Percentage of M+1	Pentacosane	43.7	45.5	51.2	46.1	36.6	41.4	40.4	38.9	41.3	57.3	37.0	50.8	45.4	57.8	45.0
	Heptacosane	38.2	41.8	50.3	44.9	41.1	44.0	39.9	41.4	37.3	45.2	33.7	35.3	27.7	38.5	44.9
Percentage of M+2	Pentacosane	15.2	20.8	27.3	16.9	11.4	18.2	15.3	15.0	12.8	23.7	13.6	16.6	16.9	28.9	17.9
	Heptacosane	14.6	17.0	29.8	12.9	11.0	16.0	18.7	17.7	11.9	20.3	13.7	12.9	19.2	21.6	22.3
Average M+n pentacosane and heptacosane values		27.9	31.3	39.7	30.2	25.0	29.9	28.6	28.2	25.8	36.6	24.5	28.9	27.3	36.7	32.5

0
0-20
20-40
40-60
60-80
80-100
100+

Figure E.24: Data sheet showing page one, repeat three of the basic yellow data for *Myrmica rubra* at a dye concentration of 2 g/L.

Basic yellow 2g / L Repeat 3		*Myrmica rubra*									
		YM_23_16	YM_23_17	YM_23_18	YM_23_19	YM_23_20	YM_23_21	YM_23_22	YM_23_23	YM_23_24	YM_23_25
Abundance of M	Pentacosane	13871	13868	20464	28440	21256	13867	6904	25312	17272	27168
	Heptacosane	5116	4368	6918	10937	6846	3570	2317	10912	5794	12479
Abundance of M+1	Pentacosane	5950	7537	8865	12426	10917	8459	2502	12716	7335	11717
	Heptacosane	1523	2465	3046	4207	2821	2164	435	5301	2411	5166
Abundance of M+2	Pentacosane	2140	4418	2858	4703	4959	3538	862	4217	2745	5597
	Heptacosane	819	828	1246	1765	1405	775	285	1579	785	1973
Percentage of M+1	Pentacosane	42.9	54.3	43.3	43.7	51.4	61.0	36.2	50.2	42.5	43.1
	Heptacosane	29.8	56.4	44.0	38.5	41.2	60.6	18.8	48.6	41.6	41.4
Percentage of M+2	Pentacosane	15.4	31.9	14.0	16.5	23.3	25.5	12.5	16.7	15.9	20.6
	Heptacosane	16.0	19.0	18.0	16.1	20.5	21.7	12.3	14.5	13.5	15.8
Average M+n pentacosane and heptacosane values		26.0	40.4	29.8	28.7	34.1	42.2	20.0	32.5	28.4	30.2

0
0-20
20-40
40-60
60-80
80-100
100+
30.6%

Figure E.25: Data sheet showing page two, repeat three of the basic yellow data for *Myrmica rubra* at a dye concentration of 2 g/L.

Basic yellow 5g / L Repeat 1		*Myrmica rubra*																
		YM_51_1	YM_51_2	YM_51_3	YM_51_4	YM_51_5	YM_51_6	YM_51_7	YM_51_8	YM_51_9	YM_51_10	YM_51_11	YM_51_12	YM_51_13	YM_51_14	YM_51_15	YM_51_16	YM_51_17
Abundance of M	Pentacosane	29088	11827	12064	5940	71852	10801	32560	26104	21976	17848	43952	37512	38576	34976	29720	53448	63000
	Heptacosane	10207	5036	3666	2623	23936	3091	12386	7764	6890	5847	13462	15222	10570	11791	8295	22600	22840
Abundance of M+1	Pentacosane	12117	4829	4767	2797	27504	4050	12214	10974	8378	6600	20824	14631	16520	13917	9372	16904	23488
	Heptacosane	3943	2054	1546	1241	10695	857	5137	2429	2582	2038	4385	5463	4715	4803	3906	7399	9387
Abundance of M+2	Pentacosane	3959	1724	1825	681	8965	1113	3055	3324	2873	1879	7395	4800	5441	4723	2644	3983	5926
	Heptacosane	1254	323	740	230	3354	467	945	967	953	493	2138	2148	1779	1249	1012	1764	2345
Percentage of M+1	Pentacosane	41.7	40.8	39.5	47.1	38.3	37.5	37.5	42.0	38.1	37.0	47.4	39.0	42.8	39.8	31.5	31.6	37.3
	Heptacosane	38.6	40.8	42.2	47.3	44.7	27.7	41.5	31.3	37.5	34.9	32.6	35.9	44.6	40.7	47.1	32.7	41.1
Percentage of M+2	Pentacosane	13.6	14.6	15.1	11.5	12.5	10.3	9.4	12.7	13.1	10.5	16.8	12.8	14.1	13.5	8.9	7.5	9.4
	Heptacosane	12.3	6.4	20.2	8.8	14.0	15.1	7.6	12.5	13.8	8.4	15.9	14.1	16.8	10.6	12.2	7.8	10.3
Average M+n pentacosane and heptacosane values		26.5	25.7	29.2	28.7	27.4	22.7	24.0	24.6	25.6	31.0	28.2	25.4	29.6	26.2	24.9	19.9	24.5

0
0-20
20-40
40-60
60-80
80-100
100+
26.1%

Figure E.26: Data sheet showing repeat one of the basic yellow data for *Myrmica rubra* at a dye concentration of 5 g/L.

Basic yellow 5g / L Repeat 2		*Myrmica rubra*														
		YM_52_1	YM_52_2	YM_52_3	YM_52_4	YM_52_5	YM_52_6	YM_52_7	YM_52_8	YM_52_9	YM_52_10	YM_52_11	YM_52_12	YM_52_13	YM_52_14	YM_52_15
Abundance of M	Pentacosane	32464	18632	30528	16061	21800	52040	35976	21856	30968	38288	31576	31752	43376		25216
	Heptacosane	9406	7386	13058	5588	6150	21528	12779	6222	11538	15266	9778	12552	16007		8198
Abundance of M+1	Pentacosane	14111	7349	11457	10408	7806	17536	20744	11657	13240	20072	18936	11766	15788		12396
	Heptacosane	3616	3990	5196	2436	2400	8918	7383	3164	5853	7218	5543	5188	5427		3722
Abundance of M+2	Pentacosane	6377	2765	5036	4288	2305	6181	10119	5143	3923	10915	9200	3941	5043		5363
	Heptacosane	2106	1347	1872	1175	1223	2646	3285	1080	2536	3334	2227	1578	1976		1568
Percentage of M+1	Pentacosane	43.5	39.4	37.5	64.8	35.8	33.7	57.7	53.3	42.8	52.4	60.0	37.1	36.4		49.2
	Heptacosane	38.4	54.0	39.8	43.6	39.0	41.4	57.8	50.9	50.7	47.3	56.7	41.3	33.9		45.4
Percentage of M+2	Pentacosane	19.6	14.8	16.5	26.7	10.6	11.9	28.1	23.5	12.7	28.5	29.1	12.4	11.6		21.3
	Heptacosane	22.4	18.2	14.3	21.0	19.9	12.3	25.7	17.4	22.0	21.8	22.8	12.6	12.3		19.1
Average M+n pentacosane and heptacosane values		31.0	31.6	27.0	39.0	26.3	24.8	42.3	36.3	32.0	37.5	42.1	25.8	23.6		33.7

0
0-20
20-40
40-60
60-80
80-100
100+

Figure E.27: Data sheet showing page one, repeat two of the basic yellow data for *Myrmica rubra* at a dye concentration of 5 g/L.

Basic yellow 5g / L Repeat 2		*Myrmica rubra*										
		YM_52_16	YM_52_17	YM_52_18	YM_52_19	YM_52_20	YM_52_21	YM_52_22	YM_52_23	YM_52_24	YM_52_25	YM_52_26
Abundance of M	Pentacosane	35608	45344	42488	11795	13791	17808	22784	12708	24928	34928	25848
	Heptacosane	10723	13294	12728	3583	4587	5663	4419	5171	9069	10123	8540
Abundance of M+1	Pentacosane	19224	19560	18632	5876	6022	8804	8517	7055	12944	19096	12198
	Heptacosane	4652	6982	6862	1411	2596	2611	2104	2035	3812	4471	3865
Abundance of M+2	Pentacosane	9645	8941	7521	1919	3087	4411	3056	2056	5461	8391	5199
	Heptacosane	2951	2386	2781	534	1222	1228	733	999	2255	2172	1836
Percentage of M+1	Pentacosane	54.0	43.1	43.9	49.8	43.7	49.4	37.4	55.5	51.9	54.7	47.2
	Heptacosane	43.4	52.5	53.9	39.4	56.6	46.1	47.6	39.4	42.0	44.2	45.3
Percentage of M+2	Pentacosane	27.1	19.7	17.7	16.3	22.4	24.8	13.4	16.2	21.9	24.0	20.1
	Heptacosane	27.5	17.9	21.8	14.9	26.6	21.7	16.6	19.3	24.9	21.5	21.5
Average M+n pentacosane and heptacosane values		38.0	33.3	34.3	30.1	37.3	35.5	28.7	32.6	35.2	36.1	33.5

0	
0-20	
20-40	
40-60	
60-80	
80-100	
100+	
	33.1%

Figure E.28: Data sheet showing page two, repeat two of the basic yellow data for *Myrmica rubra* at a dye concentration of 5 g/L.

Basic yellow 5g / L Repeat 3		*Myrmica rubra*														
		YM_53_1	YM_53_2	YM_53_3	YM_53_4	YM_53_5	YM_53_6	YM_53_7	YM_53_8	YM_53_9	YM_53_10	YM_53_11	YM_53_12	YM_53_13	YM_53_14	YM_53_15
Abundance of M	Pentacosane	21048	26760	21672	22552	15023	12600	35424	28368	38480	23912	21168	25120	25680	36448	30104
	Heptacosane	6290	6823	4663	5641	3507	3315	12391	9444	9948	8021	7322	5961	7422	7803	7315
Abundance of M+1	Pentacosane	8820	12629	11181	10567	8039	6157	12798	11358	18088	12311	12050	12126	14025	16768	16342
	Heptacosane	2466	2884	2950	2565	2260	1788	4710	3763	6141	3708	3851	3771	2276	3992	3362
Abundance of M+2	Pentacosane	2996	3862	3788	3270	4175	4195	4729	4284	7779	6922	6034	4204	4889	5888	7847
	Heptacosane	899	919	947	871	505	774	1754	1563	2002	1415	2219	1593	1461	1976	1497
Percentage of M+1	Pentacosane	41.9	47.2	51.6	46.9	53.5	48.9	36.1	40.0	47.0	51.5	56.9	48.3	54.6	46.0	54.3
	Heptacosane	39.2	42.3	63.3	45.5	64.4	53.9	38.0	39.8	61.7	46.2	52.6	63.3	30.7	51.2	46.0
Percentage of M+2	Pentacosane	14.2	14.4	17.5	14.5	27.8	33.3	13.3	15.1	20.2	28.9	28.5	16.7	19.0	16.2	26.1
	Heptacosane	14.3	13.5	20.3	15.4	14.4	23.3	14.2	16.6	20.1	17.6	30.3	26.7	19.7	25.3	20.5
Average M+n pentacosane and heptacosane values		27.4	29.3	38.2	30.6	40.0	39.9	25.4	27.9	37.3	36.1	42.1	38.7	31.0	34.7	36.7

0
0-20
20-40
40-60
60-80
80-100
100+

Figure E.29: Data sheet showing page one, repeat three of the basic yellow data for *Myrmica rubra* at a dye concentration of 5 g/L.

Basic yellow 5g / L Repeat 3		*Myrmica rubra*								
		YM_53_16	YM_53_17	YM_53_18	YM_53_19	YM_53_20	YM_53_21	YM_53_22	YM_53_23	YM_53_24
Abundance of M	Pentacosane	37408	15813	31992	26080	21936	23144	26744	39696	26800
	Heptacosane	12280	3908	7366	5293	5899	7060	7727	10031	9638
Abundance of M+1	Pentacosane	21176	10345	17992	13449	12268	13175	12088	20288	13317
	Heptacosane	6066	2607	4510	2820	2502	4449	2994	5755	4686
Abundance of M+2	Pentacosane	9091	4438	6136	6710	5601	5950	5252	8841	4989
	Heptacosane	2683	1392	1482	2329	1354	1609	1190	2558	2040
Percentage of M+1	Pentacosane	56.6	65.4	56.2	51.6	55.9	56.9	45.2	51.1	49.7
	Heptacosane	49.4	66.7	61.2	53.3	42.4	63.0	38.7	57.4	48.6
Percentage of M+2	Pentacosane	24.3	28.1	19.2	25.7	25.5	25.7	19.6	22.3	18.6
	Heptacosane	21.8	35.6	20.1	44.0	23.0	22.8	15.4	25.5	21.2
Average M+n pentacosane and heptacosane values		38.0	49.0	39.2	43.6	36.7	42.1	29.7	39.1	34.5

0
0-20
20-40
40-60
60-80
80-100
100+

36.1%

Figure E.30: Data sheet showing page two, repeat three of the basic yellow data for *Myrmica rubra* at a dye concentration of 5 g/L.

Basic yellow Dye only		*Myrmica rubra*										
		YM_DY_1	YM_DY_2	YM_DY_3	YM_DY_4	YM_DY_5	YM_DY_6	YM_DY_7	YM_DY_8	YM_DY_9	YM_DY_10	YM_DY_11
Abundance of M	Pentacosane	10800	38944	44320	74224		79928	67472	75552	25376	39536	58200
	Heptacosane	4180	9127	10560	22800		20928	16600	19392	6790	10254	16065
Abundance of M+1	Pentacosane	3504	10725	12280	19952		22496	19112	21024	6394	11735	17184
	Heptacosane	1553	2833	3825	6594		7422	5427	4862	2161	3115	4594
Abundance of M+2	Pentacosane	531	1214	1743	2456		2425	2064	2926	889	1427	2445
	Heptacosane	325	270	563	1116		860	715	818	288	494	1009
Percentage of M+1	Pentacosane	32.4	27.5	27.7	26.9		28.1	28.3	27.8	25.2	29.7	29.5
	Heptacosane	37.2	31.0	36.2	28.9		35.5	32.7	25.1	31.8	30.4	28.6
Percentage of M+2	Pentacosane	4.9	3.1	3.9	3.3		3.0	3.1	3.9	3.5	3.6	4.2
	Heptacosane	7.8	3.0	5.3	4.9		4.1	4.3	4.2	4.2	4.8	6.3
Average M+n pentacosane and heptacosane values		20.6	16.2	18.3	16.0		17.7	17.1	15.2	16.2	17.1	17.2

0
0-20
20-40
40-60
60-80
80-100
100+
17.2%

Figure E.31: Data sheet showing the raw data for the basic yellow, dye only control for *Myrmica rubra*

Sodium [$^{13}C_2$]acetate only		*Myrmica rubra*										
		M_SA_1	M_SA_2	M_SA_3	M_SA_4	M_SA_5	M_SA_6	M_SA_7	M_SA_8	M_SA_9	M_SA_10	M_SA_11
Abundance of M	Pentacosane	28944	38720	49840	34848	49904	37816	54952	10347	4693	13424	8188
	Heptacosane	8662	14255	9358	14612	17696	11985	15738	2692	1230	3343	2513
Abundance of M+1	Pentacosane	11259	19696	21440	16728	22448	15700	24448	4898	3412	5696	3302
	Heptacosane	2823	6318	4428	5631	6215	4244	6435	1261	801	1620	601
Abundance of M+2	Pentacosane	4071	7730	8390	7831	6972	6010	9121	2019	1241	1825	1029
	Heptacosane	1183	2825	1570	2629	2092	1999	1924	706	513	589	255
Percentage of M+1	Pentacosane	38.9	50.9	43.0	48.0	45.0	41.5	44.5	47.3	72.7	42.4	40.3
	Heptacosane	32.6	44.3	47.3	38.5	35.1	35.4	40.9	46.8	65.1	48.5	23.9
Percentage of M+2	Pentacosane	14.1	20.0	16.8	22.5	14.0	15.9	16.6	19.5	26.4	13.6	12.6
	Heptacosane	13.7	19.8	16.8	18.0	11.8	16.7	12.2	26.2	41.7	17.6	10.1
Average M+n pentacosane and heptacosane values		24.8	33.7	31.0	31.8	26.5	27.4	28.6	35.0	51.5	30.5	21.7

0
0-20
20-40
40-60
60-80
80-100
100+
31.1%

Figure E.32: Data sheet showing the raw data for the sodium [$^{13}C_2$]acetate control for *Myrmica rubra*

Rhodamine B 2g / L Repeat 1		*Formica fusca*							
		RF_21_1	RF_21_2	RF_21_3	RF_21_4	RF_21_5	RF_21_6	RF_21_7	RF_21_8
Abundance of M	Pentacosene	11931	4596		11931	12703	13082	7573	58640
	Heptacosene	1448	970		3529	2407	2223	1125	11688
Abundance of M+1	Pentacosene	9506	3867		8328	8592	8647	5948	43752
	Heptacosene	1004	637		2739	1489	1299	806	8013
Abundance of M+2	Pentacosene	6089	2143		6449	5336	5246	5026	26144
	Heptacosene	582	326		1833	783	806	606	4923
Percentage of M+1	Pentacosene	79.7	84.1		69.8	67.6	66.1	78.5	74.6
	Heptacosene	69.3	65.7		77.6	61.9	58.4	71.6	68.6
Percentage of M+2	Pentacosene	51.0	46.6		54.1	42.0	40.1	66.4	44.6
	Heptacosene	40.2	33.6		51.9	32.5	36.3	53.9	42.1
Average M+n pentacosene and heptacosene values		60.1	57.5		63.4	51.0	50.2	67.6	57.5

0
0-20
20-40
40-60
60-80
80-100
100+

58.2%

Figure E.33: Data sheet showing repeat one of the rhodamine B data for *Formica fusca* at a dye concentration of 2 g/L.

Rhodamine B 2g / L Repeat 2		*Formica fusca*							
		RF_22_1	RF_22_2	RF_22_3	RF_22_4	RF_22_5	RF_22_6	RF_22_7	RF_22_8
Abundance of M	Pentacosene	7762	2624		17848	9063	6044	10092	
	Heptacosene	924	493		2718	1376	1166	1590	
Abundance of M+1	Pentacosene	5860	1750		12773	6447	4234	6923	
	Heptacosene	819	345		2080	923	634	1193	
Abundance of M+2	Pentacosene	4111	1034		6036	3738	2313	3978	
	Heptacosene	511	201		1239	596	269	709	
Percentage of M+1	Pentacosene	75.5	66.7		71.6	71.1	70.1	68.6	
	Heptacosene	88.6	70.0		76.5	67.1	54.4	75.0	
Percentage of M+2	Pentacosene	53.0	39.4		33.8	41.2	38.3	39.4	
	Heptacosene	55.3	40.8		45.6	43.3	23.1	44.6	
Average M+n pentacosene and heptacosene values		68.1	54.2		56.9	55.7	46.4	56.9	

0
0-20
20-40
40-60
60-80
80-100
100+

56.4%

Figure E.34: Data sheet showing repeat two of the rhodamine B data for *Formica fusca* at a dye concentration of 2 g/L.

Rhodamine B 2g / L Repeat 3		*Formica fusca*							
		RF_23_1	RF_23_2	RF_23_3	RF_23_4	RF_22_5	RF_22_6	RF_22_7	RF_22_8
Abundance of M	Pentacosene	2114		12755	7761	12331	16332		11277
	Heptacosene	397		2480	1068	1858	2975		3553
Abundance of M+1	Pentacosene	1878		5374	5255	10955	10064		9239
	Heptacosene	235		1894	800	1700	1941		2502
Abundance of M+2	Pentacosene	927		3258	3121	7165	6842		5415
	Heptacosene	275		1201	601	1139	1166		1247
Percentage of M+1	Pentacosene	88.8		42.1	67.7	88.8	61.6		81.9
	Heptacosene	59.2		76.4	74.9	91.5	65.2		70.4
Percentage of M+2	Pentacosene	43.9		25.5	40.2	58.1	41.9		48.0
	Heptacosene	69.3		48.4	56.3	61.3	39.2		35.1
Average M+n pentacosene and heptacosene values		65.3		48.1	59.8	74.9	52.0		58.9

0
0-20
20-40
40-60
60-80
80-100
100+
59.8%

Figure E.35: Data sheet showing repeat three of the rhodamine B data for *Formica fusca* at a dye concentration of 2 g/L.

Rhodamine B Dye only		*Formica fusca*																	
		RF_DY_1	RF_DY_2	RF_DY_3	RF_DY_4	RF_DY_5	RF_DY_6	RF_DY_7	RF_DY_8	RF_DY_9	RF_DY_10	RF_DY_11	RF_DY_12	RF_DY_13	RF_DY_14	RF_DY_15	RF_DY_16	RF_DY_17	RF_DY_18
Abundance of M	Pentacosene		287232	364032	245120	152448	312256	129968	374016	180928	376064	232512	332288	271872	126288	140032	264960	339584	310400
	Heptacosene		162432	224448	117888	71952	180480	71816	217344	81672	276608	135232	158272	128072	60416	56000	171008	291712	11708
Abundance of M+1	Pentacosene		73920	96736	65528	39488	82544	34944	99200	48600	103032	61152	85544	76208	33312	37392	72512	88872	84216
	Heptacosene		48680	65384	35248	20040	53096	20624	65032	24616	77528	41304	46336	36936	17656	16259	51048	85176	3501
Abundance of M+2	Pentacosene		9699	12589	8802	5043	10722	4604	12187	5614	13005	7849	11900	9222	5264	4540	9211	10855	11098
	Heptacosene		6532	9580	5072	3290	7483	3103	8377	3392	11034	5541	6631	5368	2865	1972	6981	11905	411
Percentage of M+1	Pentacosene		25.7	26.6	26.7	25.9	26.4	26.9	26.5	26.9	27.4	26.3	25.7	28.0	26.4	26.7	27.4	26.2	27.1
	Heptacosene		30.0	29.1	29.9	27.9	29.4	28.7	29.9	30.1	28.0	30.5	29.3	28.8	29.2	29.0	29.9	29.2	29.9
Percentage of M+2	Pentacosene		3.4	3.5	3.6	3.3	3.4	3.5	3.3	3.1	3.5	3.4	3.6	3.4	4.2	3.2	3.5	3.2	3.6
	Heptacosene		4.0	4.3	4.3	4.6	4.1	4.3	3.9	4.2	4.0	4.1	4.2	4.2	4.7	3.5	4.1	4.1	3.5
Average M+n pentacosene and heptacosene values			15.8	15.9	16.1	15.4	15.9	15.9	15.9	16.1	15.7	16.1	15.7	16.1	16.1	15.6	16.2	15.7	16.0

0
0-20
20-40
40-60
60-80
80-100
100+

15.9%

Figure E.36: Data sheet showing the raw data for the rhodamine B, dye only control for *Formica fusca*

Sodium [$^{13}C_2$]acetate only		*Formica fusca*								
		FSA_1	FSA_2	FSA_3	FSA_4	FSA_5	FSA_6	FSA_7	FSA_8	FSA_9
Abundance of M	Pentacosene	335232	243136	322944	535232	235456	413760	469376	461632	476160
	Heptacosene	65040	35960	65088	101904	50720	95584	87720	95824	95696
Abundance of M+1	Pentacosene	160384	133824	185536	285440	153088	235776	245824	279488	306304
	Heptacosene	39224	28328	39256	58848	36680	51928	48912	61840	78064
Abundance of M+2	Pentacosene	68784	59752	83048	131456	93232	107072	112280	136832	157952
	Heptacosene	12916	12439	18472	29608	17272	33792	29760	35880	37160
Percentage of M+1	Pentacosene	47.8	55.0	57.5	53.3	65.0	57.0	52.4	60.5	64.3
	Heptacosene	60.3	78.8	60.3	57.7	72.3	54.3	55.8	64.5	81.6
Percentage of M+2	Pentacosene	20.5	24.6	25.7	24.6	39.6	25.9	23.9	29.6	33.2
	Heptacosene	19.9	34.6	28.4	29.1	34.1	35.4	33.9	37.4	38.8
Average M+n pentacosene and heptacosene values		37.1	48.2	43.0	41.2	52.7	43.1	41.5	48.0	54.5

0
0-20
20-40
40-60
60-80
80-100
100+

Figure E.37: Data sheet showing the raw data for the sodium [$^{13}C_2$]acetate control for *Formica fusca*

Rhodamine B 1g / L Repeat 1		*Myrmica rubra*					
		RM_13_1	RM_13_2	RM_13_3	RM_13_4	RM_13_5	RM_13_6
Abundance of M	Pentacosane	43911	18681	27301	19747	44768	26125
	Heptacosane	20112	11310	11652	7965	9925	7761
Abundance of M+1	Pentacosane	14854	10487	10535	7186	18244	8034
	Heptacosane	8150	4170	3486	4016	3833	3887
Abundance of M+2	Pentacosane	3868	2666	3347	2564	2897	1843
	Heptacosane	2391	783	1706	2173	632	778
Percentage of M+1	Pentacosane	33.8	56.1	38.6	36.4	40.8	30.8
	Heptacosane	40.5	36.9	29.9	50.4	38.6	50.1
Percentage of M+2	Pentacosane	8.8	14.3	12.3	13.0	6.5	7.1
	Heptacosane	11.9	6.9	14.6	27.3	6.4	10.0
Average M+n pentacosane and heptacosane values		23.8	28.6	23.9	31.8	23.1	24.5

0
0-20
20-40
40-60
60-80
80-100
100+

25.9%

Figure E.38: Data sheet showing repeat one of the rhodamine B data for *Myrmica rubra* at a dye concentration of 1 g/L.

Rhodamine B 1g / L Repeat 2		*Myrmica rubra*						
		RM_13_1	RM_13_2	RM_13_3	RM_13_4	RM_13_5	RM_13_6	RM_13_7
Abundance of M	Pentacosane	10572	3850	4251	6450	4927	3888	8886
	Heptacosane	3650	876	1011	1199	1830	1184	3140
Abundance of M+1	Pentacosane	4223	1888	1674	2491	2251	1412	3014
	Heptacosane	1397	277	357	472	852	510	1137
Abundance of M+2	Pentacosane	1331	655	543	908	867	479	897
	Heptacosane	427	170	159		267		402
Percentage of M+1	Pentacosane	39.9	49.0	39.4	38.6	45.7	36.3	33.9
	Heptacosane	38.3	31.6	35.3	39.4	46.6	43.1	36.2
Percentage of M+2	Pentacosane	12.6	17.0	12.8	14.1	17.6	12.3	10.1
	Heptacosane	11.7	19.4	15.7	0.0	14.6	0.0	12.8
Average M+n pentacosane and heptacosane values		25.6	29.3	25.8	23.0	31.1	22.9	23.3

0
0-20
20-40
40-60
60-80
80-100
100+
25.9%

Figure E.39: Data sheet showing repeat two of the rhodamine B data for *Myrmica rubra* at a dye concentration of 1 g/L.

Rhodamine B 1g / L Repeat 3		*Myrmica rubra*														
		RM_13_1	RM_13_2	RM_13_3	RM_13_4	RM_13_5	RM_13_6	RM_13_7	RM_13_8	RM_13_9	RM_13_10	RM_13_11	RM_13_12	RM_13_13	RM_13_14	RM_13_15
Abundance of M	Pentacosane	5495	2914	3790	6329	8197	5190	2813	5598	3188	7608	5705	4888	8453	10522	7163
	Heptacosane	1470	1159	2387	1660	2213	1363	1027	1667	1045	1845	3668	1539	3217	3674	3853
Abundance of M+1	Pentacosane	1739	1100	1648	2533	2989	1872	876	1922	997	2768	2112	1735	2653	4411	3003
	Heptacosane	539	327	668	651	963	420	288	529	313	705	1649	519	1077	1151	1102
Abundance of M+2	Pentacosane	688	372	391	577	1111	436		537	527	880	665	589	672	1558	915
	Heptacosane	194	150	205		319			226	196	233	521		372	385	373
Percentage of M+1	Pentacosane	31.6	37.7	43.5	40.0	36.5	36.1	31.1	34.3	31.3	36.4	37.0	35.5	31.4	41.9	41.9
	Heptacosane	36.7	28.2	28.0	39.2	43.5	30.8	28.0	31.7	30.0	38.2	45.0	33.7	33.5	31.3	28.6
Percentage of M+2	Pentacosane	12.5	12.8	10.3	9.1	13.6	8.4	0.0	9.6	16.5	11.6	11.7	12.0	7.9	14.8	12.8
	Heptacosane	13.2	12.9	8.6	0.0	14.4	0.0	0.0	13.6	18.8	12.6	14.2	0.0	11.6	10.5	9.7
Average M+n pentacosane and heptacosane values		23.5	22.9	22.6	22.1	27.0	18.8	14.8	22.3	24.1	24.7	27.0	20.3	21.1	24.6	23.2

Figure E.40: Data sheet showing repeat three of the rhodamine B data for *Myrmica rubra* at a dye concentration of 1 g/L.

Rhodamine B 2g / L Repeat 2		*Myrmica rubra*						
		RF_22_1	RF_22_2	RF_22_3	RF_22_4	RF_22_5	RF_22_6	RF_22_7
Abundance of M	Pentacosane	8363	22080	23376	21024	22528	12281	11450
	Heptacosane	3722	5289	4832	6828	6069	4186	4686
Abundance of M+1	Pentacosane	2660	9389	8968	7834	9015	4656	5042
	Heptacosane	1411	2498	2338	2939	3200	1725	1591
Abundance of M+2	Pentacosane	611	2950	3209	1717	3069	1689	2207
	Heptacosane	407	788	879	763	940	506	481
Percentage of M+1	Pentacosane	31.8	42.5	38.4	37.3	40.0	37.9	44.0
	Heptacosane	37.9	47.2	48.4	43.0	52.7	41.2	34.0
Percentage of M+2	Pentacosane	7.3	13.4	13.7	8.2	13.6	13.8	19.3
	Heptacosane	10.9	14.9	18.2	11.2	15.5	12.1	10.3
Average M+n pentacosane and heptacosane values		22.0	29.5	29.7	24.9	30.5	26.2	26.9

0
0-20
20-40
40-60
60-80
80-100
100+

27.1%

Figure E.41: Data sheet showing repeat two of the rhodamine B data for *Myrmica rubra* at a dye concentration of 2 g/L.

Rhodamine B 2g / L Repeat 3		*Myrmica rubra*	
		RM_23_1	RM_23_2
Abundance of M	Pentacosane	7081	25736
	Heptacosane	3505	7756
Abundance of M+1	Pentacosane	1941	9463
	Heptacosane	1521	2972
Abundance of M+2	Pentacosane	659	2255
	Heptacosane	254	850
Percentage of M+1	Pentacosane	27.4	36.8
	Heptacosane	43.4	38.3
Percentage of M+2	Pentacosane	9.3	8.8
	Heptacosane	7.2	11.0
Average M+n pentacosane and heptacosane values		21.8	23.7

0
0-20
20-40
40-60
60-80
80-100
100+

22.8%

Figure E.42: Data sheet showing repeat three of the rhodamine B data for *Myrmica rubra* at a dye concentration of 2 g/L.

Rhodamine B 5g / L Repeat 1		*Myrmica rubra*									
		RM_51_1	RM_51_2	RM_51_3	RM_51_4	RM_51_5	RM_51_6	RM_51_7	RM_51_8	RM_51_9	RM_51_10
Abundance of M	Pentacosane	17760	23792	17384	8883	22576	34608	11100	25416	18880	49736
	Heptacosane	4934	10071	5888	2402	8083	11231	4576	9250	7410	17368
Abundance of M+1	Pentacosane	6808	8235	6265	3548	8826	12097	3979	9428	6659	16664
	Heptacosane	2028	3484	1993	912	3024	4522	1889	2491	2845	6525
Abundance of M+2	Pentacosane	1731	1607	1784	770	2380	2671	625	2404	1785	4033
	Heptacosane	936	740	736	277	934	1307	451	1319	996	1952
Percentage of M+1	Pentacosane	38.3	34.6	36.0	39.9	39.1	35.0	35.8	37.1	35.3	33.5
	Heptacosane	41.1	34.6	33.8	38.0	37.4	40.3	41.3	26.9	38.4	37.6
Percentage of M+2	Pentacosane	9.7	6.8	10.3	8.7	10.5	7.7	5.6	9.5	9.5	8.1
	Heptacosane	19.0	7.3	12.5	11.5	11.6	11.6	9.9	14.3	13.4	11.2
Average M+n pentacosane and heptacosane values		27.0	20.8	23.2	24.5	24.7	23.6	23.2	21.9	24.1	22.6

0
0-20
20-40
40-60
60-80
80-100
100+

23.6%

Figure E.43: Data sheet showing repeat one of the rhodamine B data for *Myrmica rubra* at a dye concentration of 5 g/L.

Rhodamine B 5g / L Repeat 2		*Myrmica rubra*					
		RM_52_1	RM_52_2	RM_52_3	RM_52_4	RM_52_5	RM_52_6
Abundance of M	Pentacosane	19200	13876		14251	15829	9950
	Heptacosane	5763	3530		4927	5124	3186
Abundance of M+1	Pentacosane	6181	4538		5350	5683	2438
	Heptacosane	2110	1646		1340	1624	1042
Abundance of M+2	Pentacosane	1877	1361		1698	1905	1091
	Heptacosane	536	178		467	659	257
Percentage of M+1	Pentacosane	32.2	32.7		37.5	35.9	24.5
	Heptacosane	36.6	46.6		27.2	31.7	32.7
Percentage of M+2	Pentacosane	9.8	9.8		11.9	12.0	11.0
	Heptacosane	9.3	5.0		9.5	12.9	8.1
Average M+n pentacosane and heptacosane values		22.0	23.5		21.5	23.1	19.1

0
0-20
20-40
40-60
60-80
80-100
100+

21.8%

Figure E.44: Data sheet showing repeat two of the rhodamine B data for *Myrmica rubra* at a dye concentration of 5 g/L.

Basic yellow 5g / L Repeat 2		*Myrmica rubra*			
		RM_53_1	RM_53_2	RM_53_3	RM_53_4
Abundance of M	Pentacosane	11667	11694	15253	5032
	Heptacosane	4174	4251	5032	2557
Abundance of M+1	Pentacosane	3667	3982	3936	2751
	Heptacosane	1012	1556	2751	494
Abundance of M+2	Pentacosane	874	1089	1031	278
	Heptacosane	497	486	278	307
Percentage of M+1	Pentacosane	31.4	34.1	25.8	54.7
	Heptacosane	24.2	36.6	54.7	19.3
Percentage of M+2	Pentacosane	7.5	9.3	6.8	5.5
	Heptacosane	11.9	11.4	5.5	12.0
Average M+n pentacosane and heptacosane values		18.8	22.8	23.2	22.9

0
0-20
20-40
40-60
60-80
80-100
100+

21.9%

Figure E.45: Data sheet showing repeat three of the rhodamine B data for *Myrmica rubra* at a dye concentration of 5 g/L.

Rhodamine B Dye only		*Myrmica rubra*													
		RF_DY_1	RF_DY_2	RF_DY_3	RF_DY_4	RF_DY_5	RF_DY_6	RF_DY_7	RF_DY_8	RF_DY_9	RF_DY_10	RF_DY_11	RF_DY_12	RF_DY_13	RF_DY_14
Abundance of M	Pentacosane		287232	364032	245120	152448	312256	129968	374016	180928	376064	232512	332288	271872	126288
	Heptacosane		162432	224448	117888	71952	180480	71816	217344	81672	276608	135232	158272	128072	60416
Abundance of M+1	Pentacosane		73920	96736	65528	39488	82544	34944	99200	48600	103032	61152	85544	76208	33312
	Heptacosane		48680	65384	35248	20040	53096	20624	65032	24616	77528	41304	46336	36936	17656
Abundance of M+2	Pentacosane		9699	12589	8802	5043	10722	4604	12187	5614	13005	7849	11900	9222	5264
	Heptacosane		6532	9580	5072	3290	7483	3103	8377	3392	11034	5541	6631	5368	2865
Percentage of M+1	Pentacosane		25.7	26.6	26.7	25.9	26.4	26.9	26.5	26.9	27.4	26.3	25.7	28.0	26.4
	Heptacosane		30.0	29.1	29.9	27.9	29.4	28.7	29.9	30.1	28.0	30.5	29.3	28.8	29.2
Percentage of M+2	Pentacosane		3.4	3.5	3.6	3.3	3.4	3.5	3.3	3.1	3.5	3.4	3.6	3.4	4.2
	Heptacosane		4.0	4.3	4.3	4.6	4.1	4.3	3.9	4.2	4.0	4.1	4.2	4.2	4.7
Average M+n pentacosane and heptacosane values			15.8	15.9	16.1	15.4	15.9	15.9	15.9	16.1	15.7	16.1	15.7	16.1	16.1

0
0-20
20-40
40-60
60-80
80-100
100+

15.9%

Figure E.46: Data sheet showing the raw data for the rhodamine B, dye only control for *Myrmica rubra*

Appendix F

Images of the Fluorescence for Chapter 7

The following pages contain images which show the fluorescence of each of the samples. All images are presented in greyscale. Please note that on occasion some of the samples have missing images. This is because these samples were initially analysed using a fluorescent microplate reader and the samples discarded. The mean brightness of the image is shown above each of the numbered sample images, the lowest and highest values are also highlighted in red and green text respectively.

A study into the relationship between substrate incorporation and amount of food consumed

Figure F.1: Images showing the fluorescence of each of the samples for repeat one, 1 g/L basic yellow, *Formica lemani*

Figure F.2: Images showing the fluorescence of each of the samples for repeat two, 1 g/L basic yellow, *Formica lemani*

Figure F.3: Images showing the fluorescence of each of the samples for repeat three, 1 g/L basic yellow, *Formica lemani*

Figure F.4: Images showing the fluorescence of each of the samples for repeat one, 2 g/L basic yellow, *Formica lemani*

Figure F.5: Images showing the fluorescence of each of the samples for repeat two, 2 g/L basic yellow, *Formica lemani*

Figure F.6: Images showing the fluorescence of each of the samples for repeat three, 2 g/L basic yellow, *Formica lemani*

Figure F.7: Images showing the fluorescence of each of the samples for repeat one, 5

g/L basic yellow, *Formica lemani*

Figure F.8: Images showing the fluorescence of each of the samples for repeat three, 5 g/L basic yellow, *Formica lemani*

Figure F.10: Images showing the fluorescence of each of the samples for the basic yellow dye only control for *Formica lemani*

Figure F.9: Images showing the fluorescence of each of the samples for the sodium [$^{13}C_2$]acetate control for *Formica lemani*

Figure F.11: Images showing the fluorescence of each of the samples for repeat one, 1 g/L basic yellow, *Myrmica rubra*

Figure F.12: Images showing the fluorescence of each of the samples for repeat two, 1 g/L basic yellow, *Myrmica rubra*

Figure F.13: Images showing the fluorescence of each of the samples for repeat one, 2 g/L basic yellow, *Myrmica rubra*

Figure F.14: Images showing the fluorescence of each of the samples for repeat two, 2 g/L basic yellow, *Myrmica rubra*

Figure F.15: Images showing the fluorescence of each of the samples for repeat three, 2 g/L basic yellow, *Myrmica rubra*

Figure F.16: Images showing the fluorescence of each of the samples for repeat one, 5 g/L basic yellow, *Myrmica rubra*

Figure F.17: Images showing the fluorescence of each of the samples for repeat two, 5 g/L basic yellow, *Myrmica rubra*

Figure F.18: Images showing the fluorescence of each of the samples for repeat three, 5 g/L basic yellow, *Myrmica rubra*

Figure F.19: Images showing the fluorescence of each of the samples for the basic yellow dye only control for *Myrmica rubra*

Figure F.20: Images showing the fluorescence of each of the samples for the sodium [$^{13}C_2$]acetate control for *Myrmica rubra* when imaged using a violet exitation source and a pale yellow filter

Figure F.21: Images showing the fluorescence of each of the samples for repeat one,

2 g/L rhodamine B, *Formica fusa*

Figure F.22: Images showing the fluorescence of each of the samples for repeat two, 2 g/L rhodamine B, *Formica fusa*

Figure F.23: Images showing the fluorescence of each of the samples for repeat three, 2 g/L rhodamine B, *Formica fusa*

Figure F.24: Images showing the fluorescence of each of the samples for the rhodamine B dye only control for *Formica fusca*

Figure F.25: Images showing the fluorescence of each of the samples for the sodium [$^{13}C_2$]acetate control for *Formica fusca*

Figure F.26: Images showing the fluorescence of each of the samples for repeat one, 1 g/L rhodamine B, *Myrmica rubra*

Figure F.27: Images showing the fluorescence of each of the samples for repeat two, 1 g/L rhodamine B, *Myrmica rubra*

Figure F.28: Images showing the fluorescence of each of the samples for repeat three, 1 g/L rhodamine B, *Myrmica rubra*

Figure F.29: Images showing the fluorescence of each of the samples for repeat two, 2 g/L rhodamine B, *Myrmica rubra*

Figure F.30: Images showing the fluorescence of each of the samples for repeat three, 2 g/L rhodamine B, *Myrmica rubra*

Figure F.31: Images showing the fluorescence of each of the samples for repeat one, 5 g/L rhodamine B, *Myrmica rubra*

Figure F.32: Images showing the fluorescence of each of the samples for repeat two, 5 g/L rhodamine B, *Myrmica rubra*

Figure F.33: Images showing the fluorescence of each of the samples for repeat three, 5 g/L rhodamine B, *Myrmica rubra*

Figure F.34: Images showing the fluorescence of each of the samples for the rhodamine B dye only control for *Myrmica rubra*

Figure F.35: Images showing the fluorescence of each of the samples for the sodium [$^{13}C_2$]acetate control for *Myrmica rubra* when imaged using a blue-green exitation

source and an orange filter

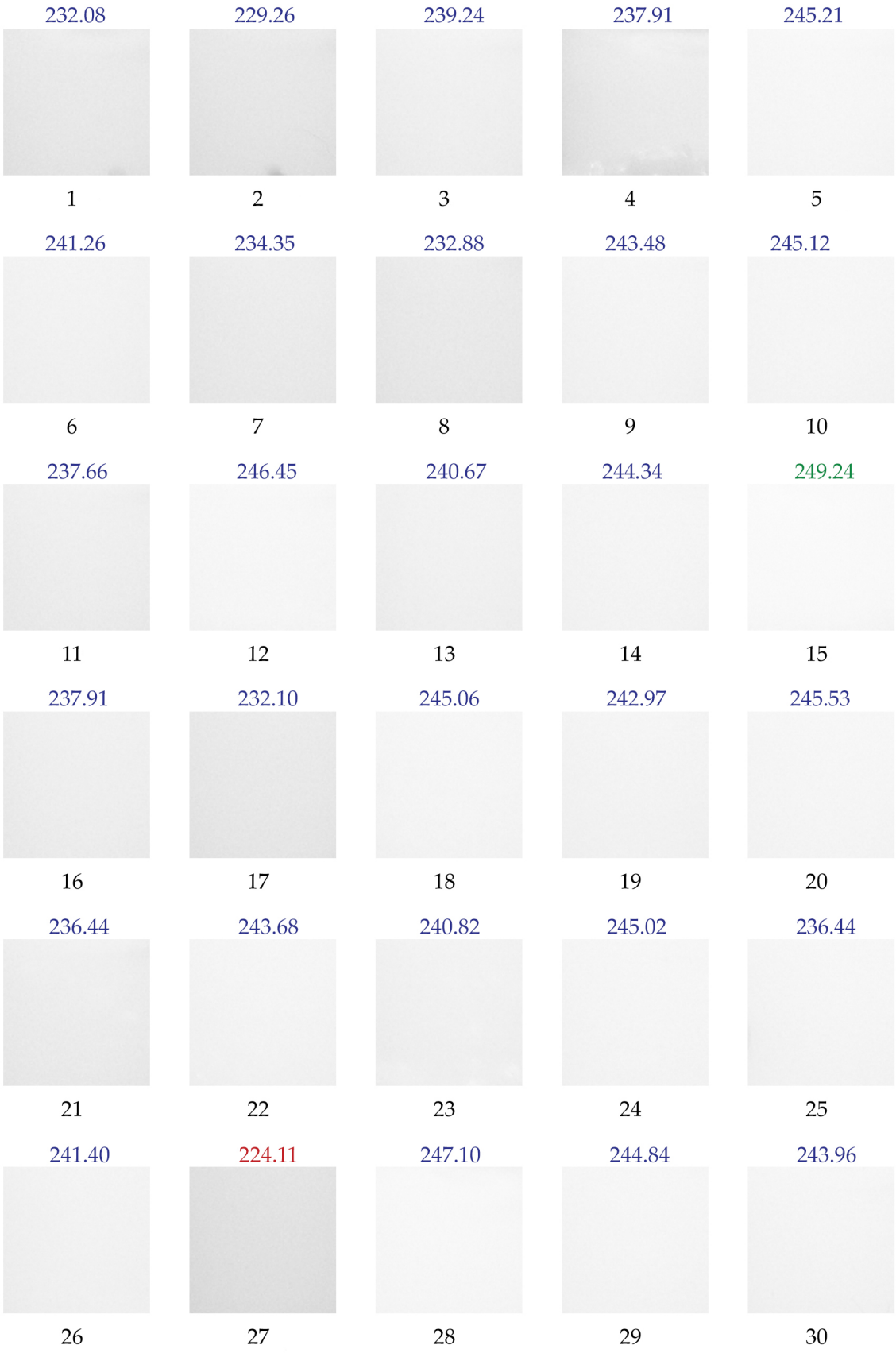


Figure F.1: Images showing the fluorescence of each of the samples for repeat one, 1 g/L basic yellow, *Formica lemani*

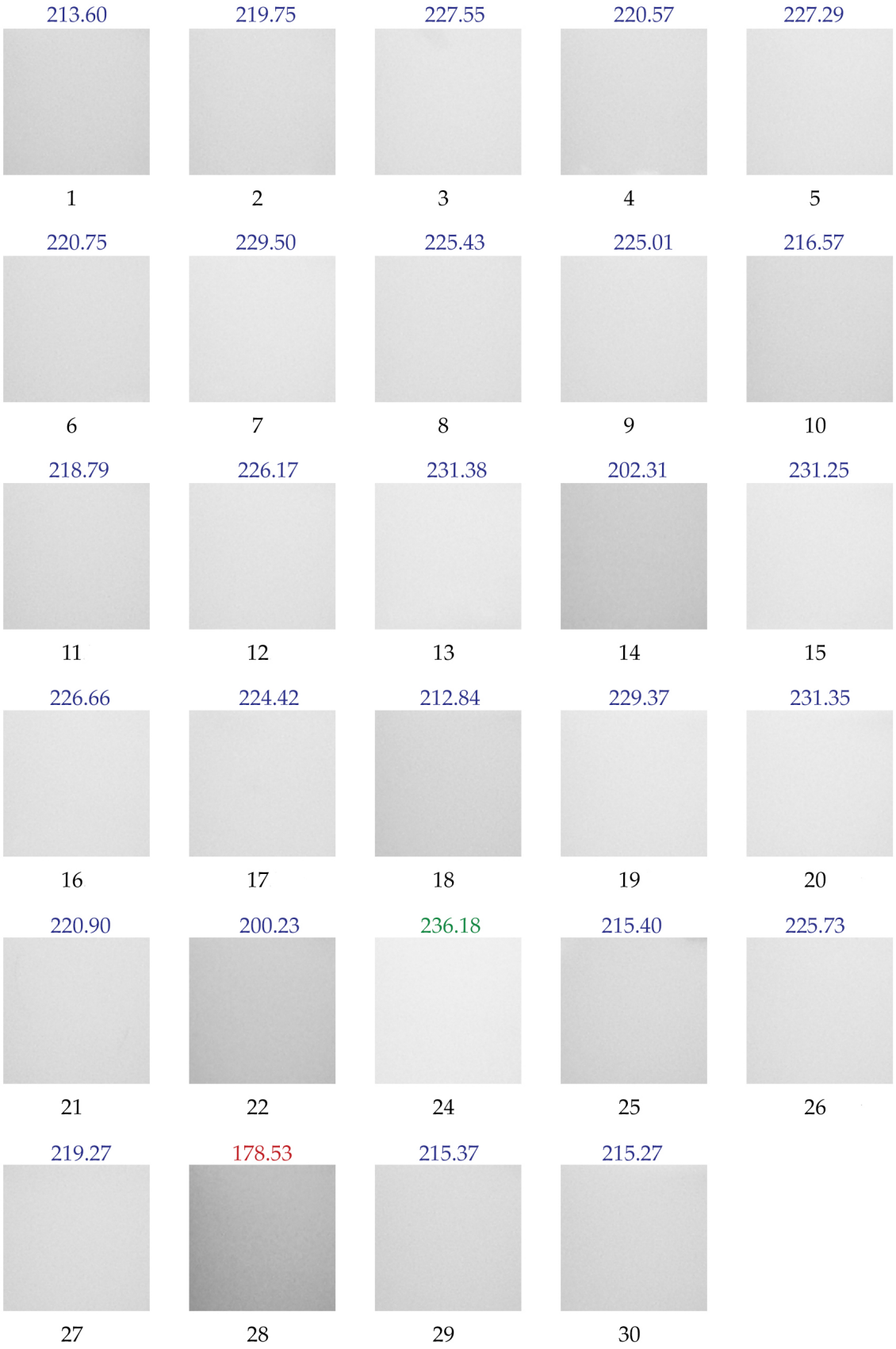


Figure F.2: Images showing the fluorescence of each of the samples for repeat two, 1 g/L basic yellow, *Formica lemani*

Figure F.3: Images showing the fluorescence of each of the samples for repeat three, 1 g/L basic yellow, *Formica lemani*

Figure F.4: Images showing the fluorescence of each of the samples for repeat one, 2 g/L basic yellow, *Formica lemani*

Figure F.5: Images showing the fluorescence of each of the samples for repeat two, 2 g/L basic yellow, *Formica lemani*

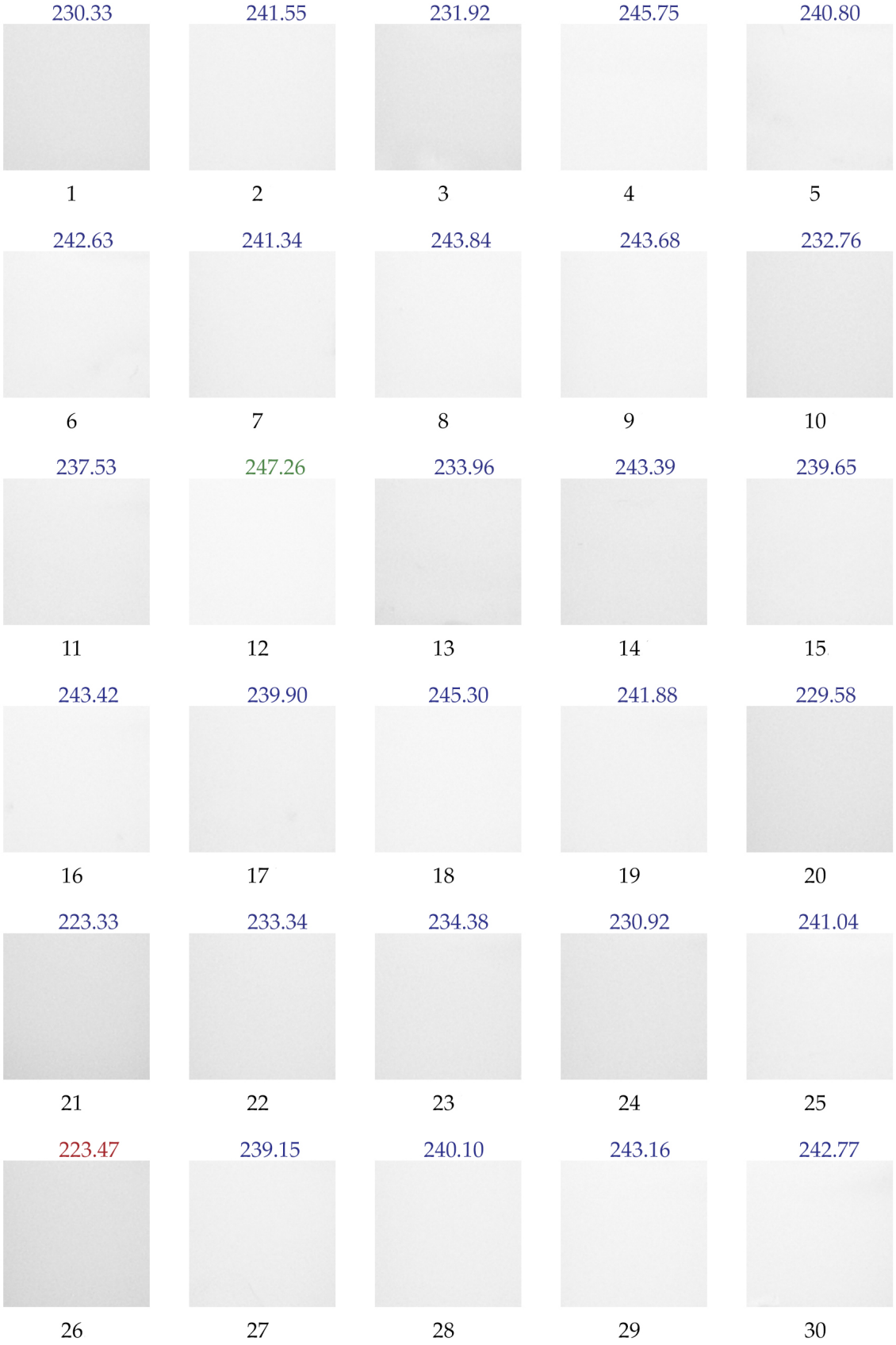


Figure F.6: Images showing the fluorescence of each of the samples for repeat three, 2 g/L basic yellow, *Formica lemani*

Figure F.7: Images showing the fluorescence of each of the samples for repeat one, 5 g/L basic yellow, *Formica lemani*

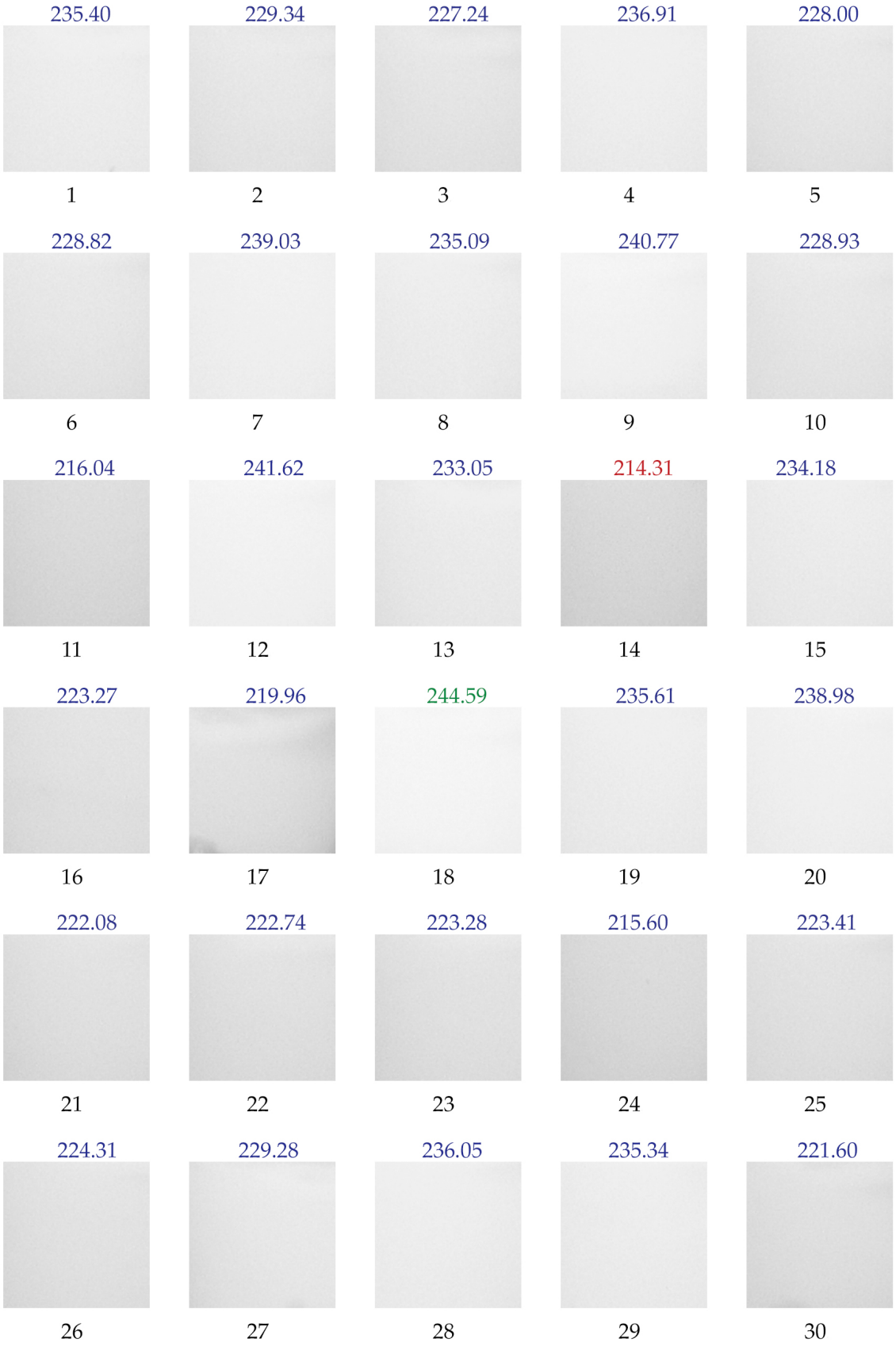


Figure F.8: Images showing the fluorescence of each of the samples for repeat three, 5 g/L basic yellow, *Formica lemani*

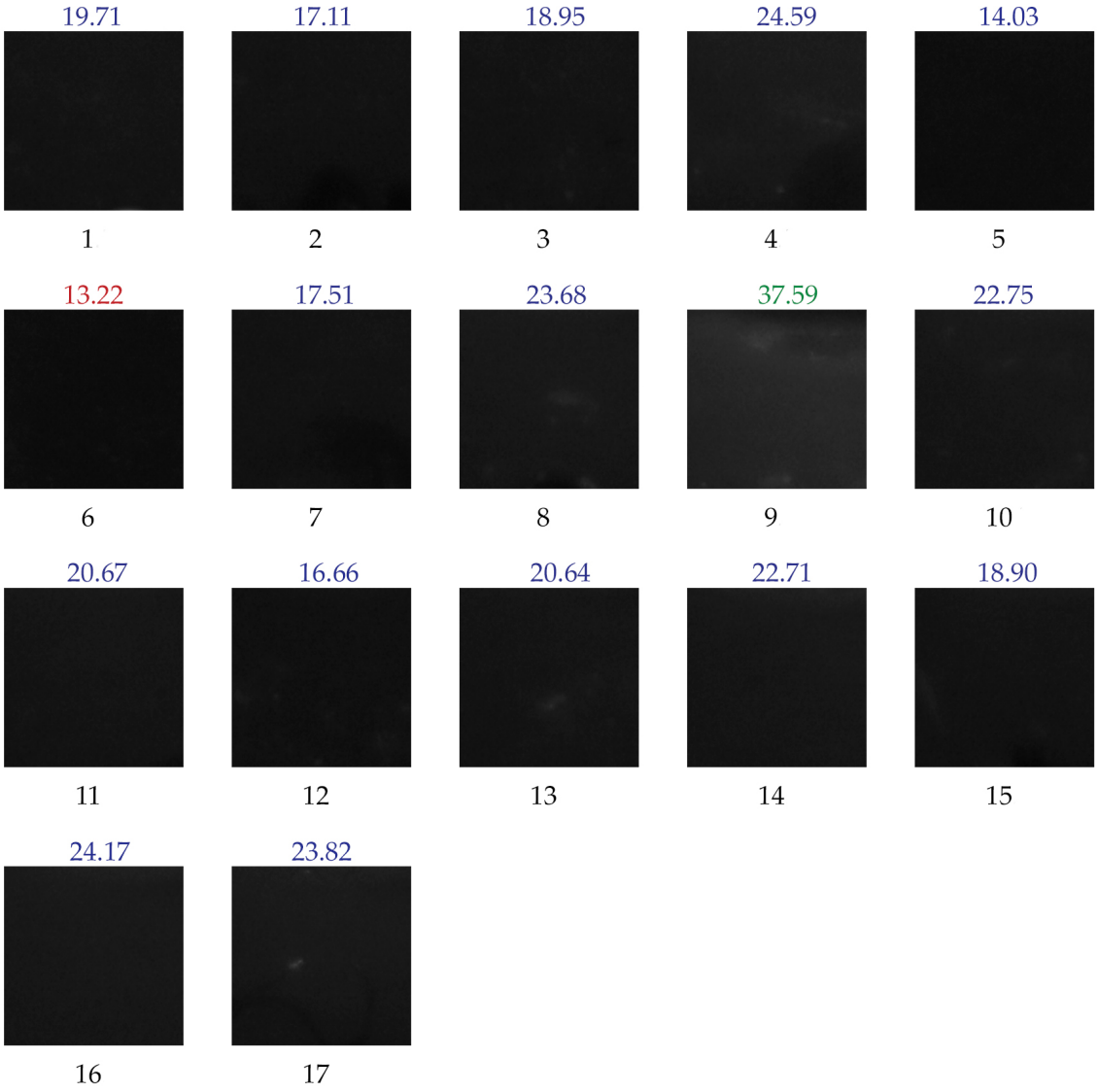


Figure F.9: Images showing the fluorescence of each of the samples for the sodium [$^{13}C_2$]acetate control for *Formica lemani*

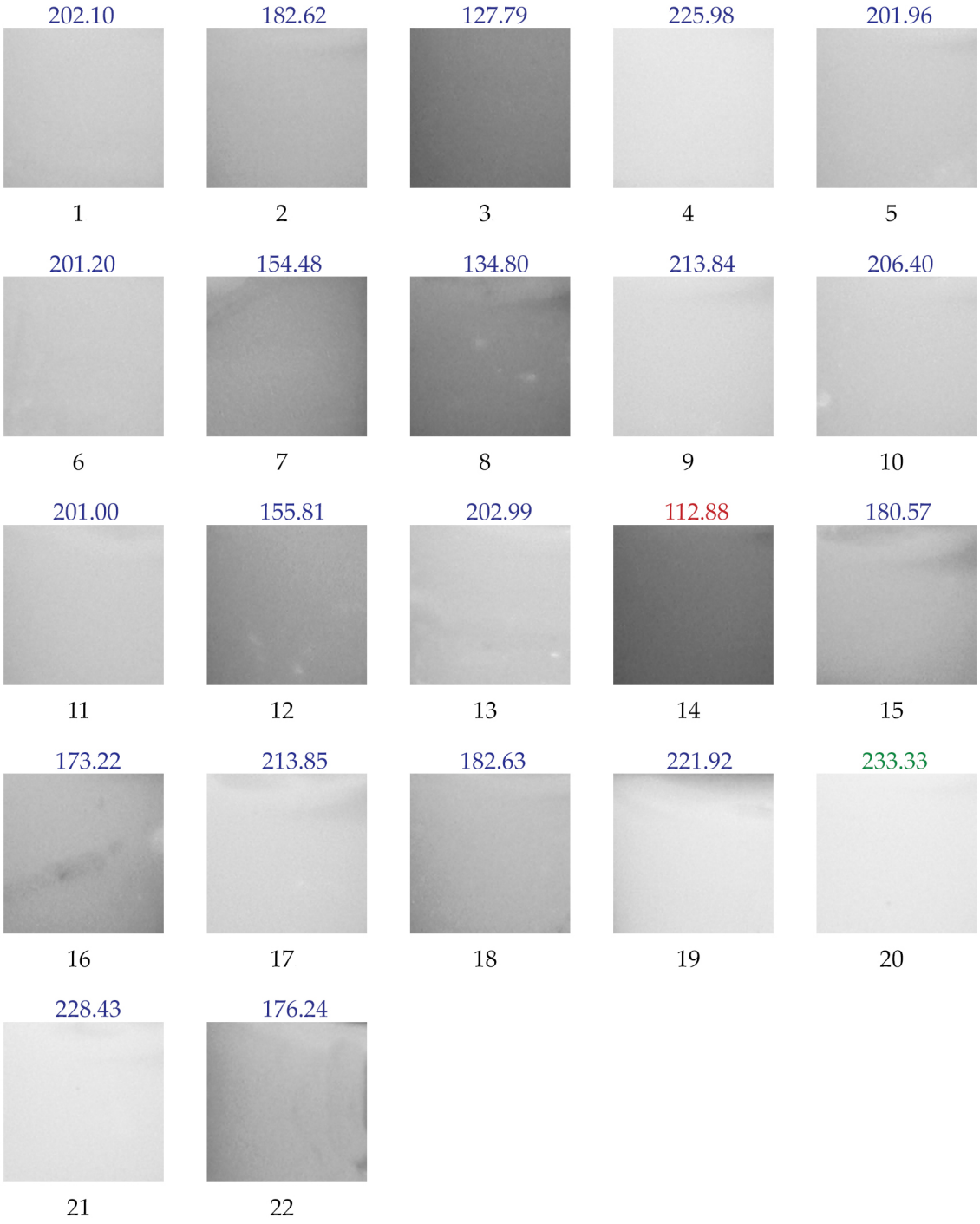


Figure F.10: Images showing the fluorescence of each of the samples for the basic yellow dye only control for *Formica lemani*

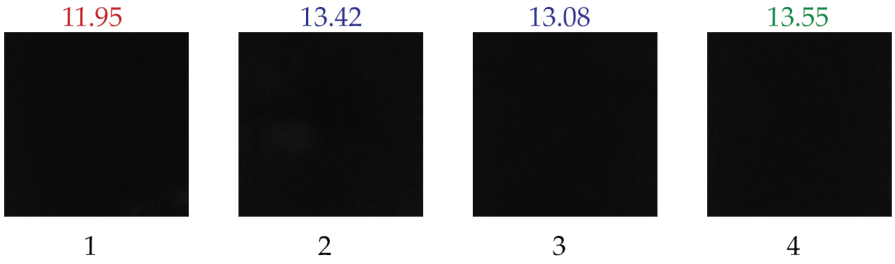


Figure F.11: Images showing the fluorescence of each of the samples for repeat one, 1 g/L basic yellow, *Myrmica rubra*

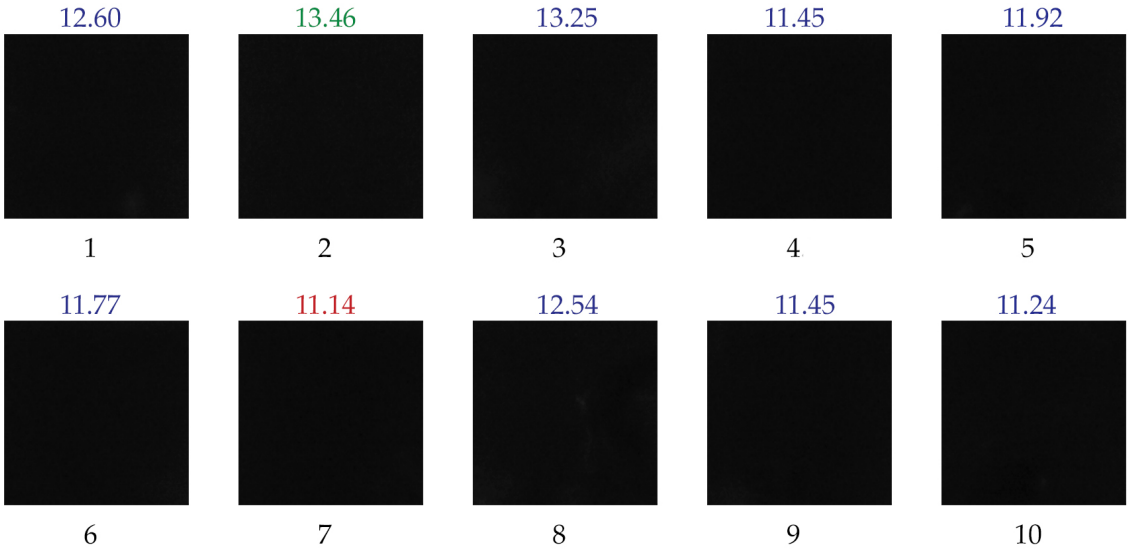


Figure F.12: Images showing the fluorescence of each of the samples for repeat two, 1 g/L basic yellow, *Myrmica rubra*

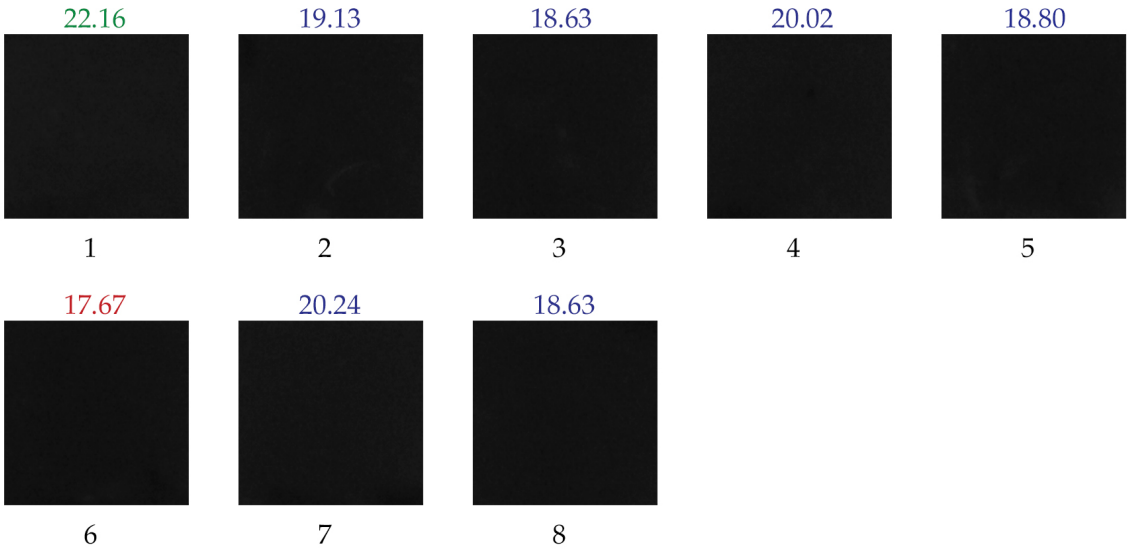


Figure F.13: Images showing the fluorescence of each of the samples for repeat one, 2 g/L basic yellow, *Myrmica rubra*

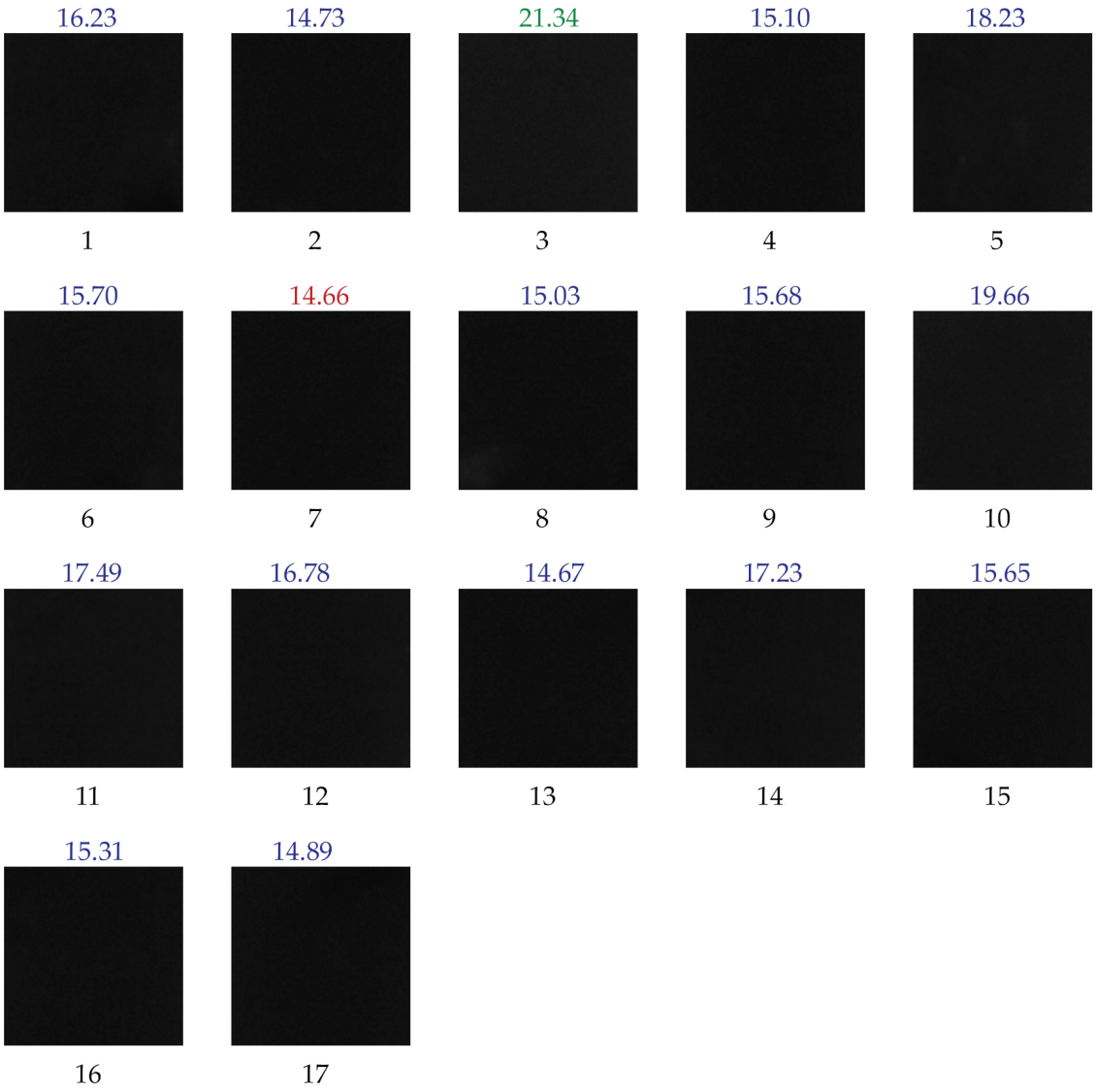


Figure F.14: Images showing the fluorescence of each of the samples for repeat two, 2 g/L basic yellow, *Myrmica rubra*

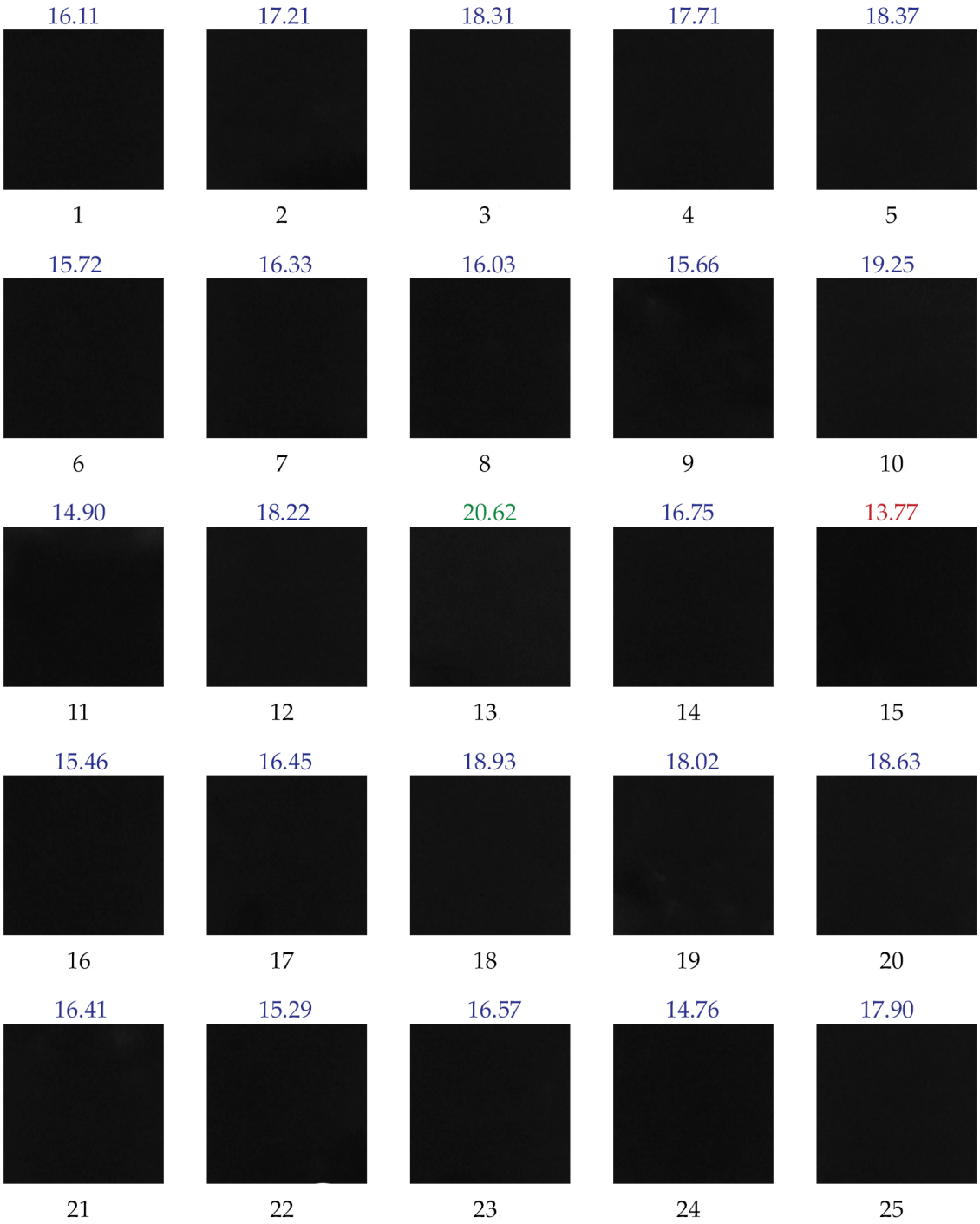


Figure F.15: Images showing the fluorescence of each of the samples for repeat three, 2 g/L basic yellow, *Myrmica rubra*

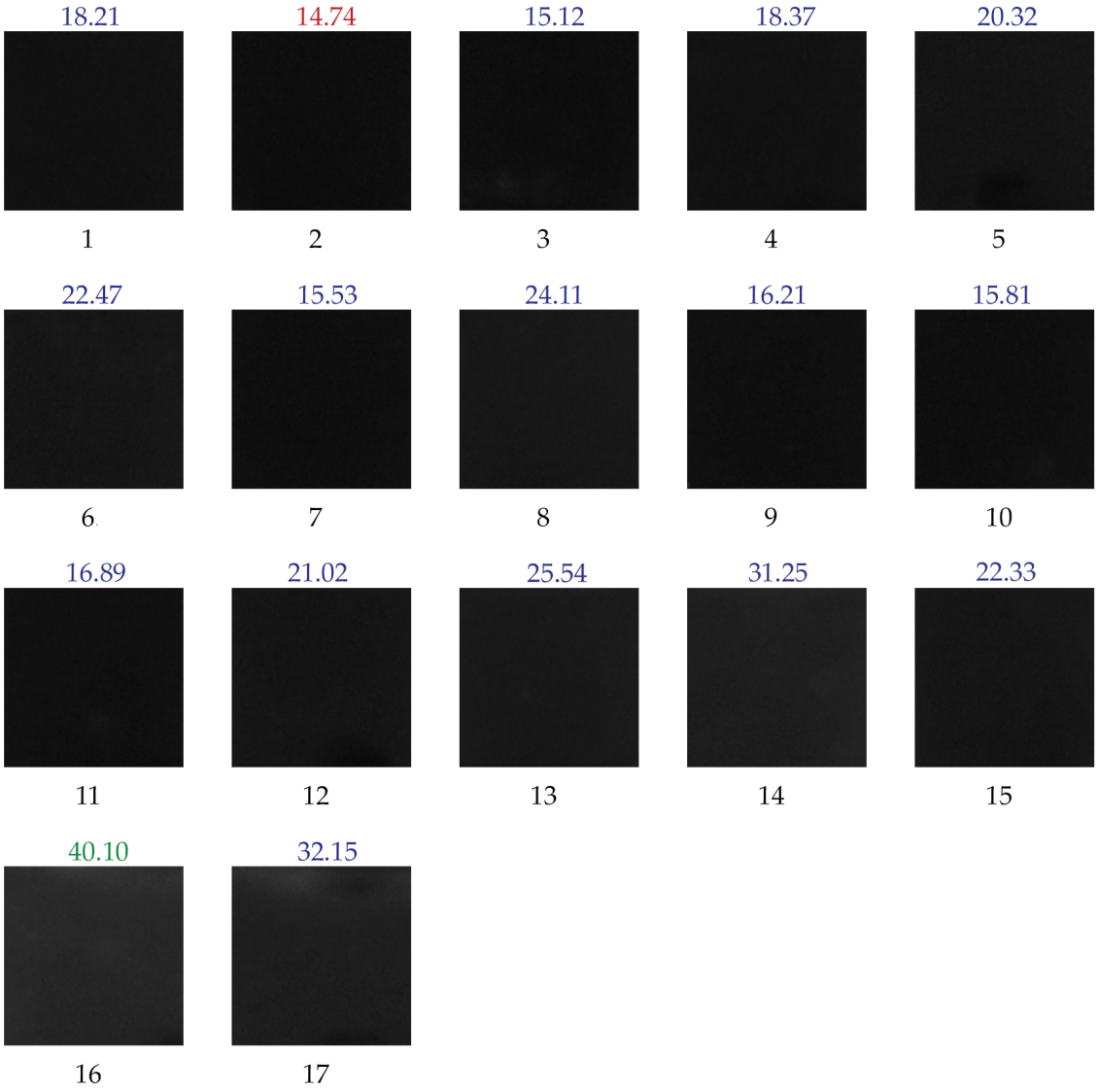


Figure F.16: Images showing the fluorescence of each of the samples for repeat one, 5 g/L basic yellow, *Myrmica rubra*

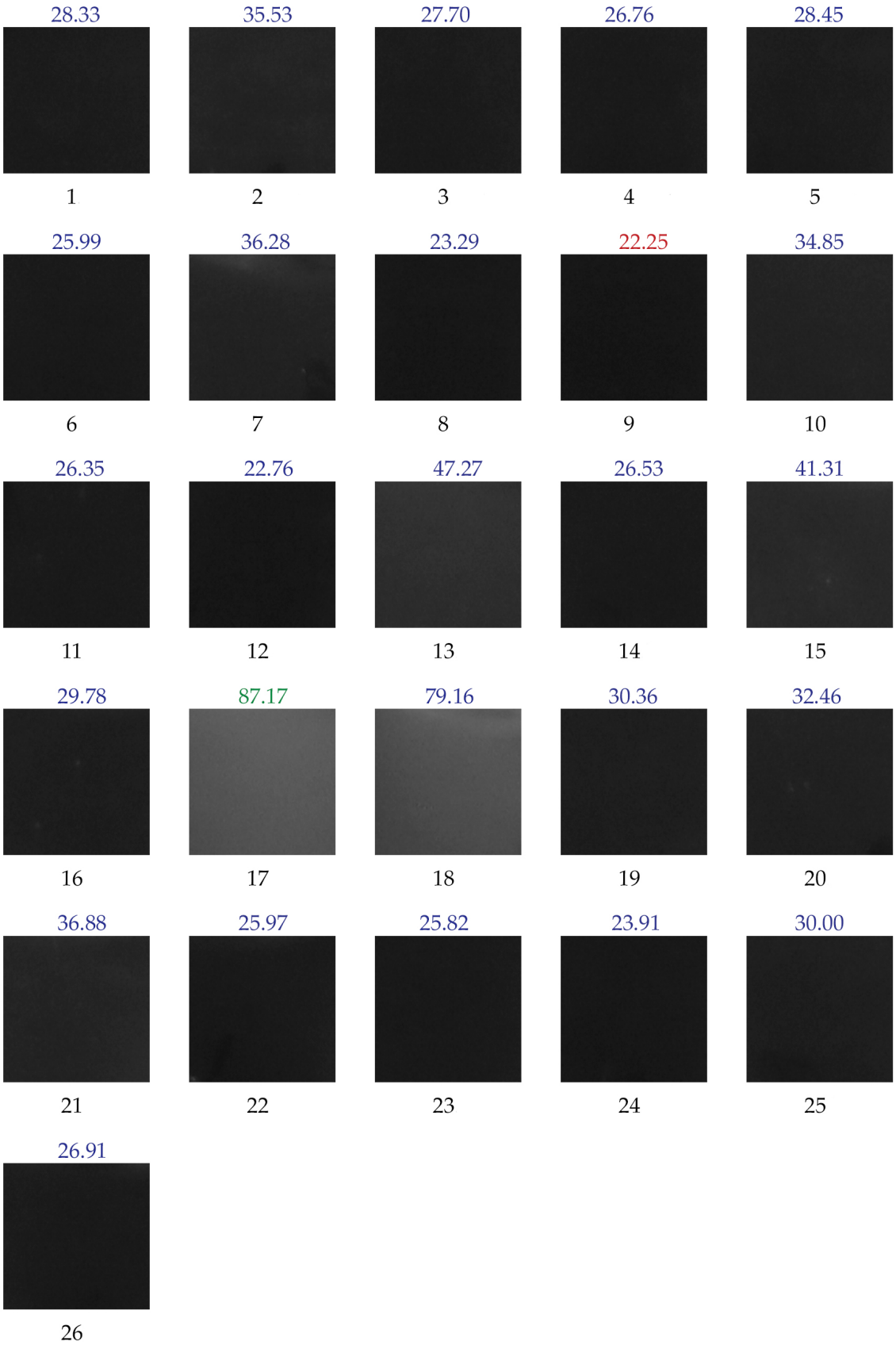


Figure F.17: Images showing the fluorescence of each of the samples for repeat two, 5 g/L basic yellow, *Myrmica rubra*

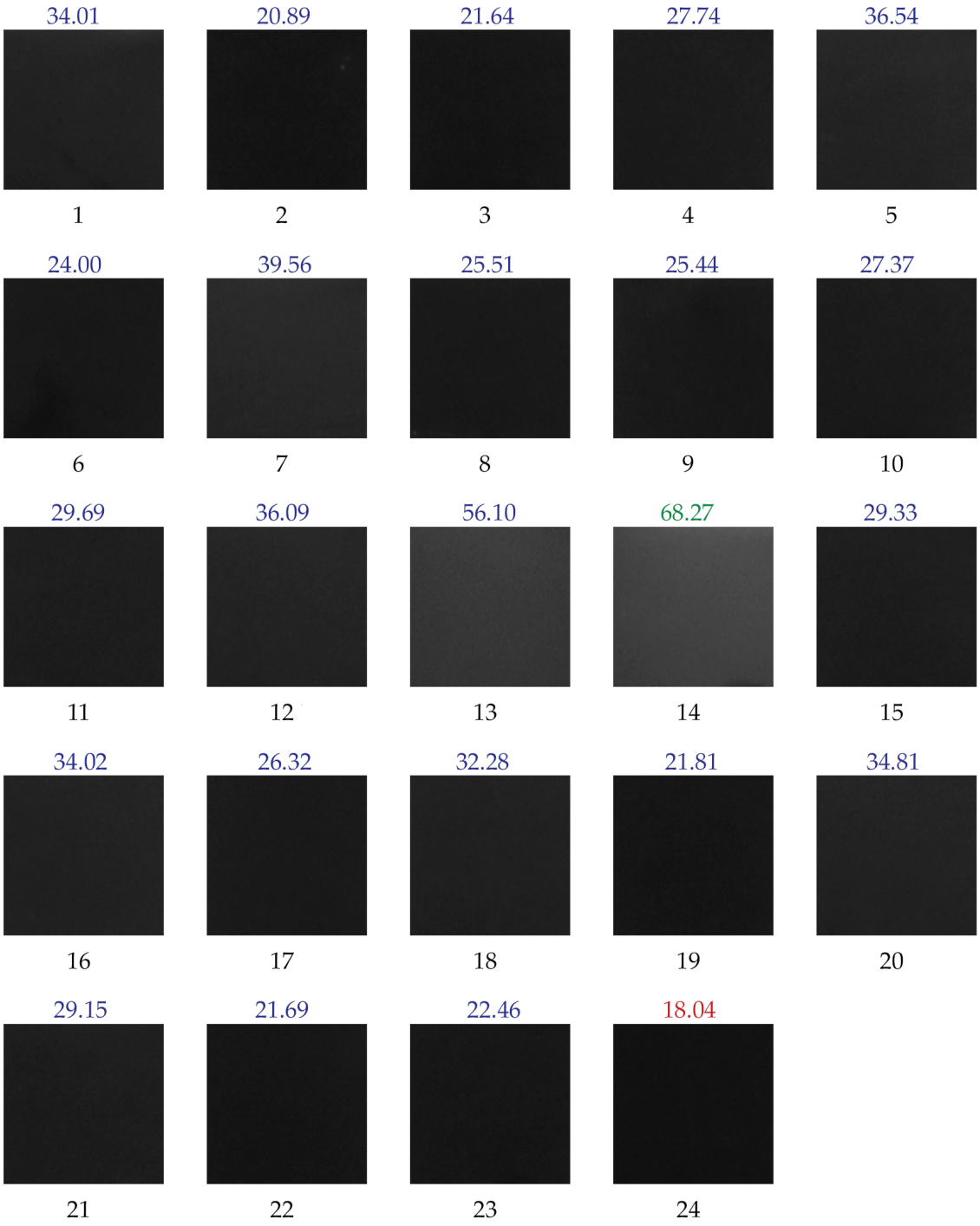


Figure F.18: Images showing the fluorescence of each of the samples for repeat three, 5 g/L basic yellow, *Myrmica rubra*

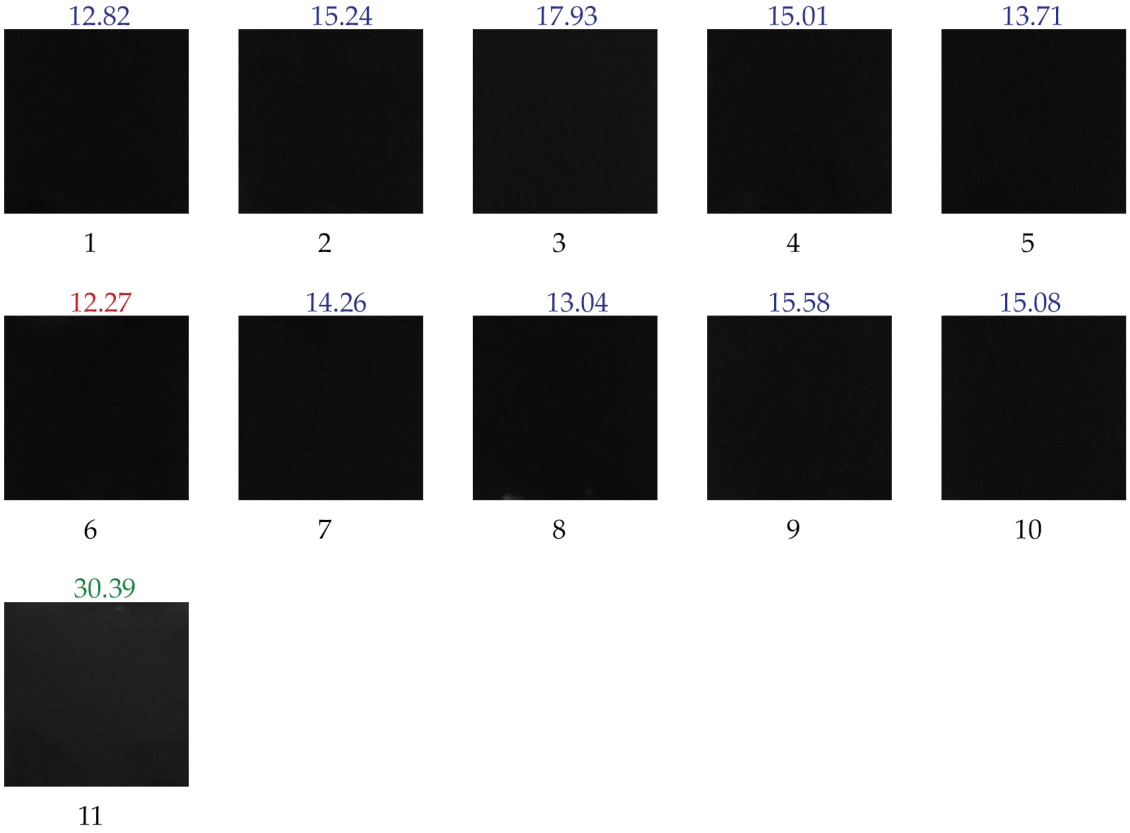


Figure F.19: Images showing the fluorescence of each of the samples for the basic yellow dye only control for *Formica lemani*

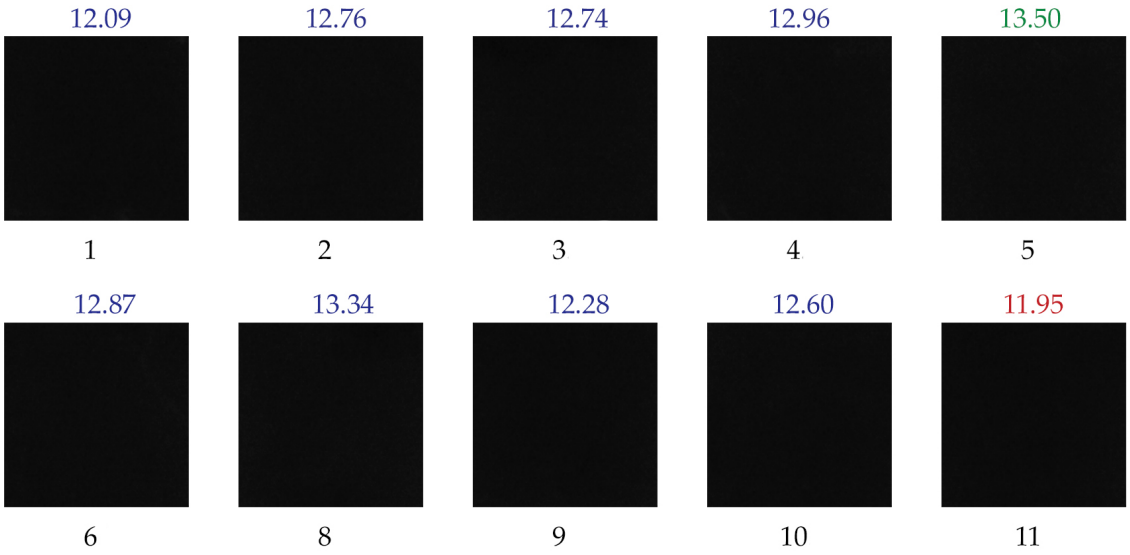


Figure F.20: Images showing the fluorescence of each of the samples for the sodium [$^{13}C_2$]acetate control for *Myrmica rubra*
when imaged using a violet excitation source and a pale yellow filter

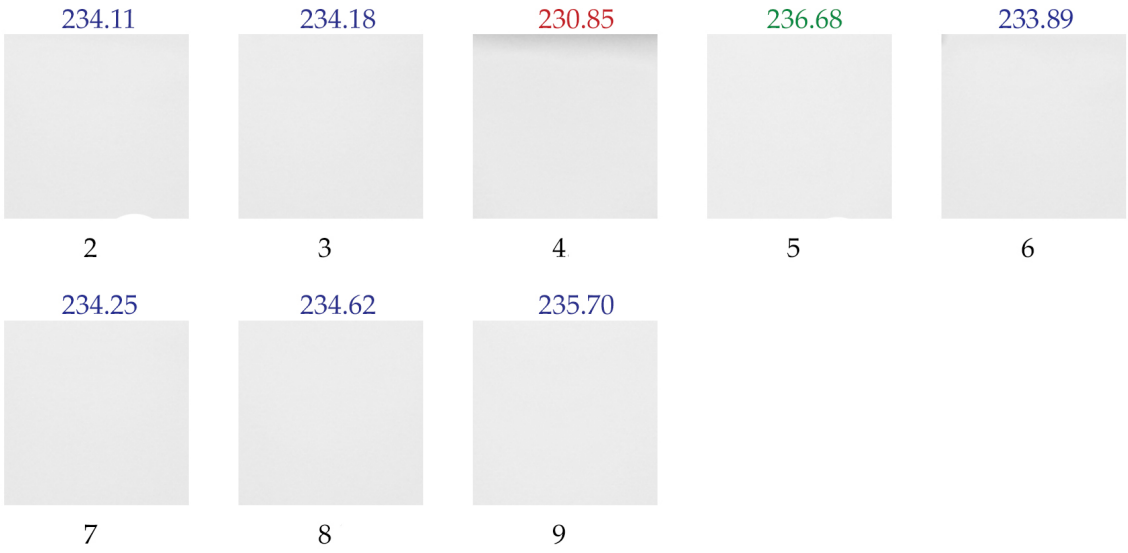


Figure F.21: Images showing the fluorescence of each of the samples for repeat one, 2 g/L basic yellow, *Formica fusca*

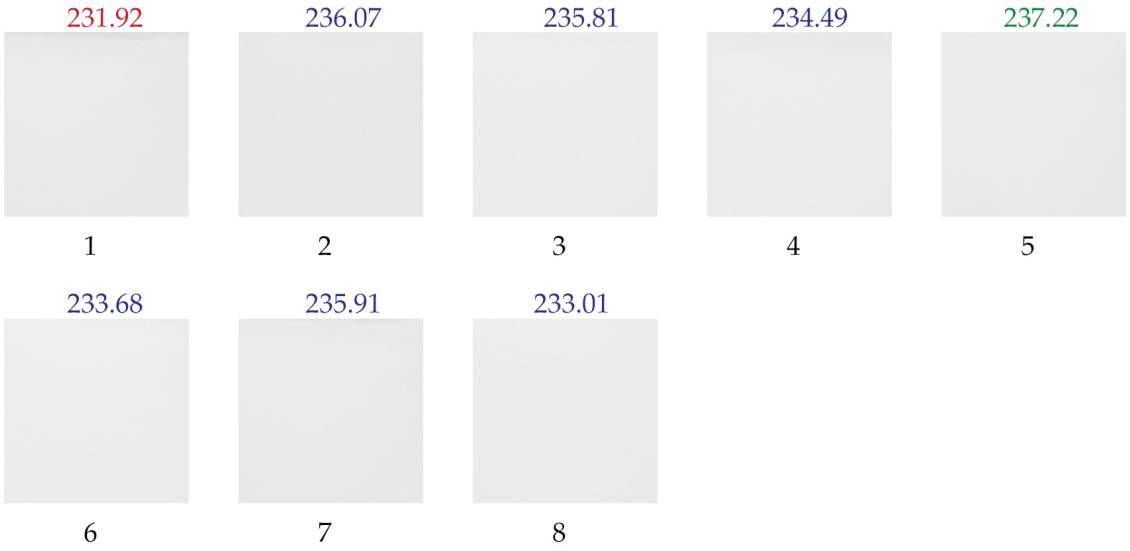


Figure F.22: Images showing the fluorescence of each of the samples for repeat two, 2 g/L basic yellow, *Formica fusa*

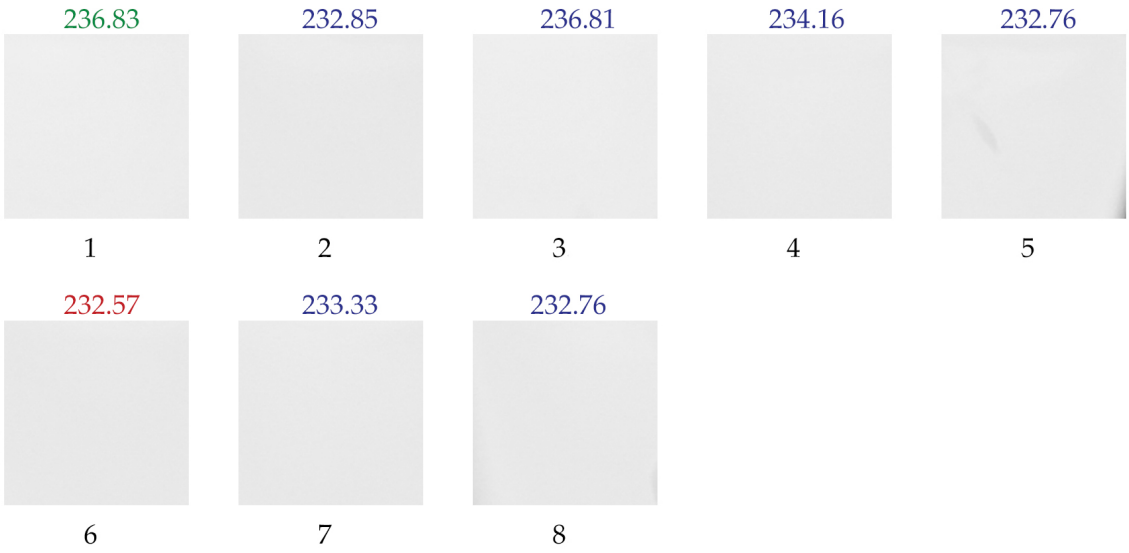


Figure F.23: Images showing the fluorescence of each of the samples for repeat three, 2 g/L basic yellow, *Formica fusca*

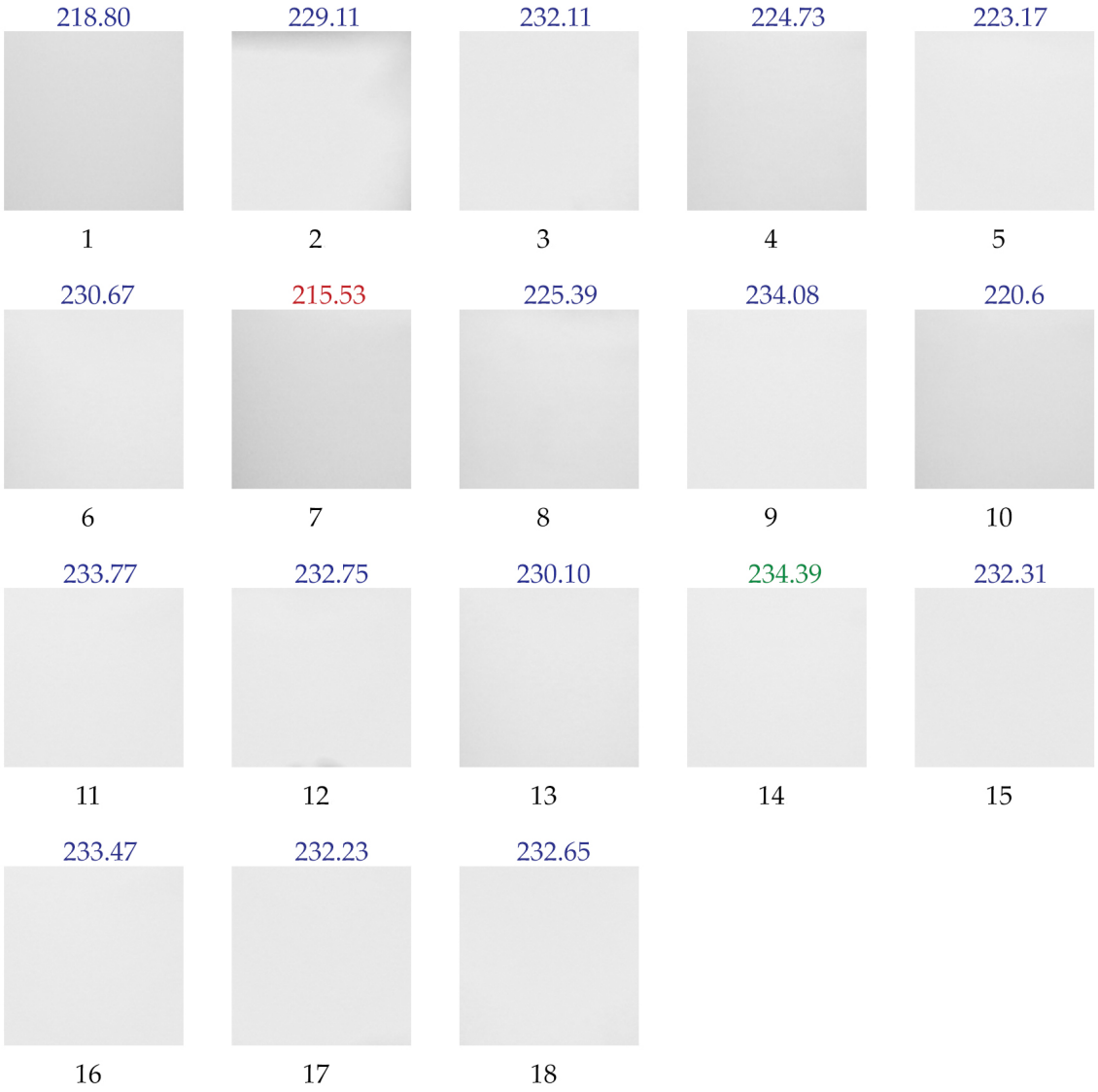


Figure F.24: Images showing the fluorescence of each of the samples for the rhodamine B dye only control for *Formica fusca*

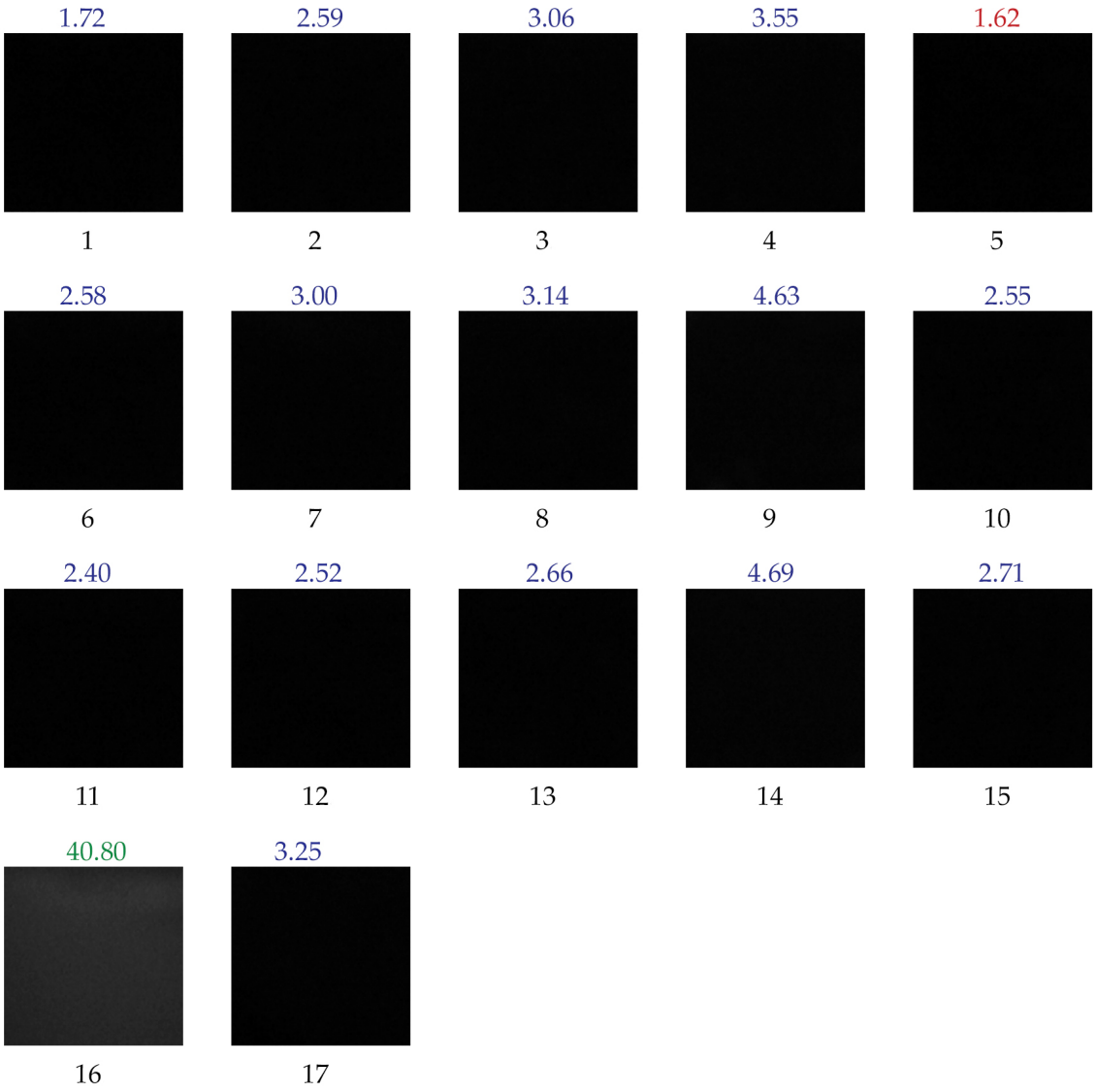


Figure F.25: Images showing the fluorescence of each of the samples for the sodium [$^{13}C_2$]acetate control for *Formica fusca*

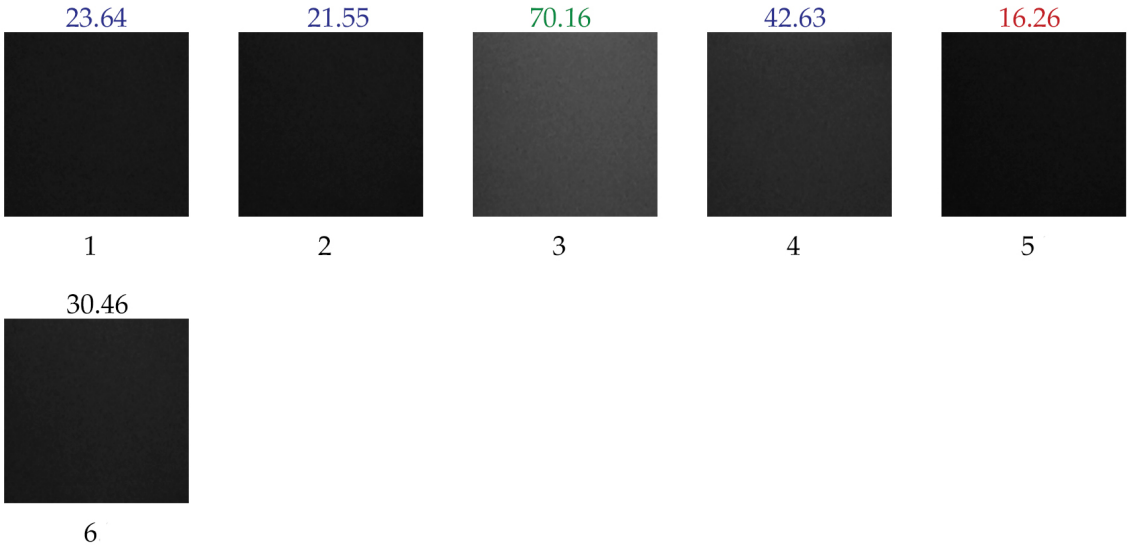


Figure F.26: Images showing the fluorescence of each of the samples for repeat one, 1 g/L rhodamine B, *Myrmica rubra*

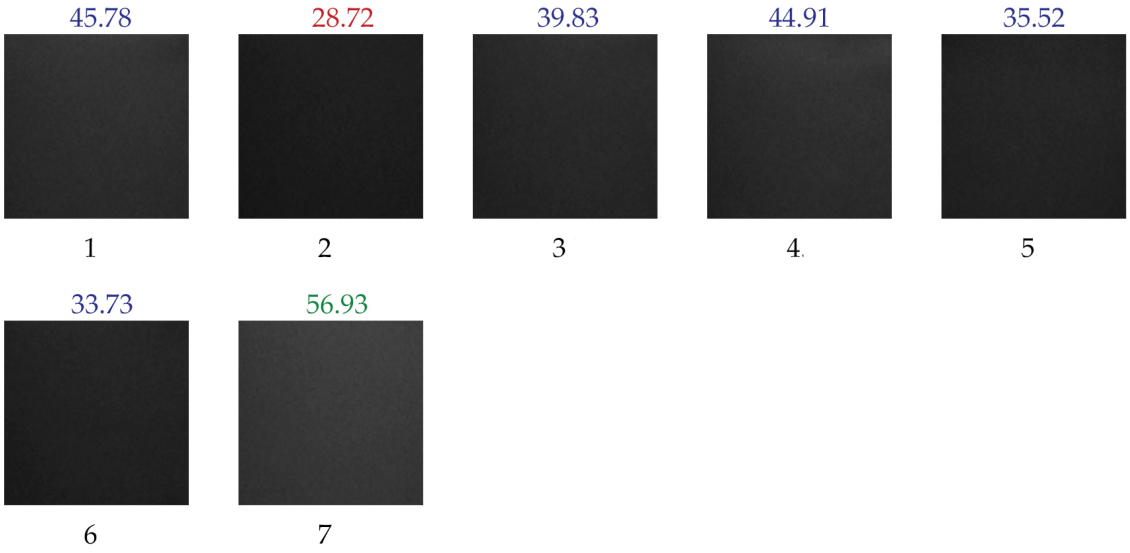


Figure F.27: Images showing the fluorescence of each of the samples for repeat two, 1 g/L rhodamine B, *Myrmica rubra*

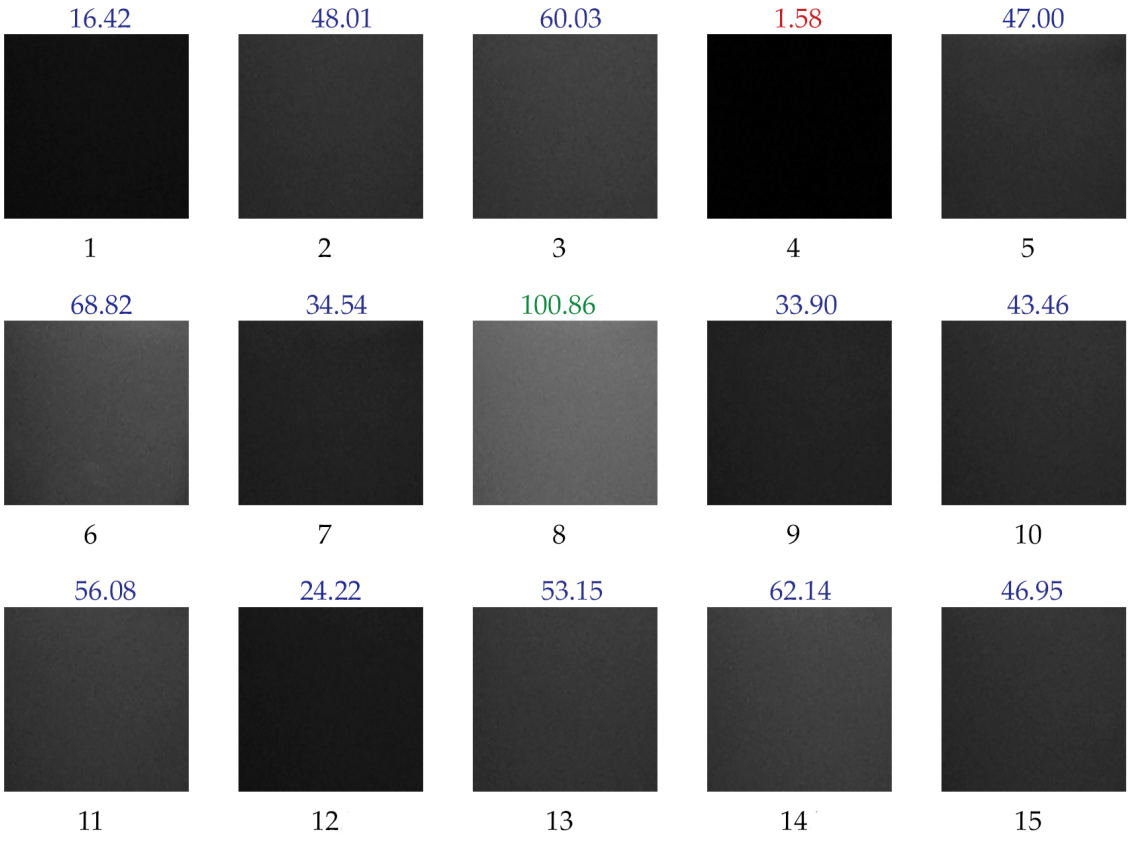


Figure F.28: Images showing the fluorescence of each of the samples for repeat two, 1 g/L rhodamine B, *Myrmica rubra*

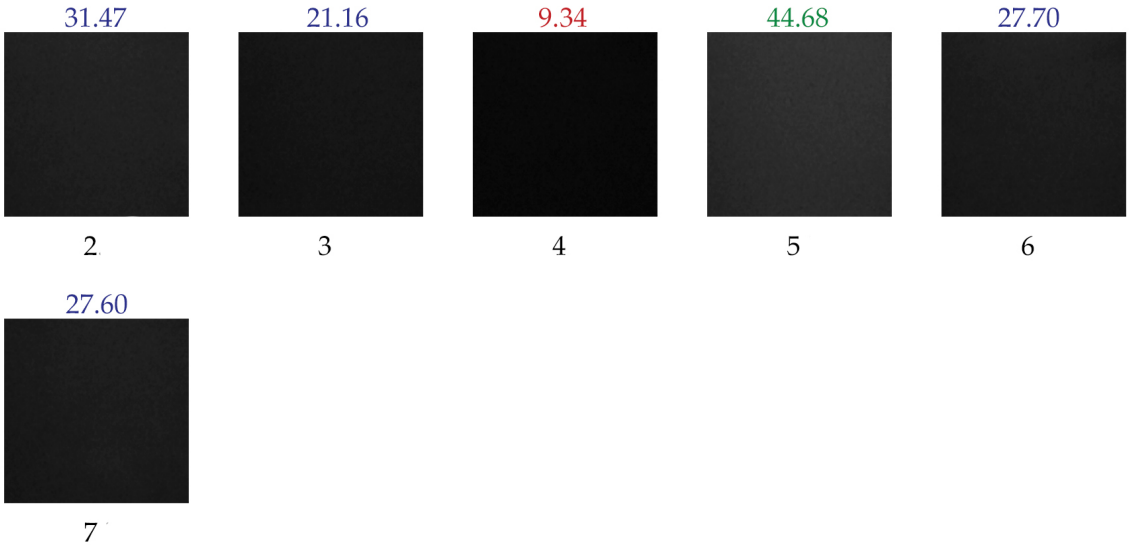


Figure F.29: Images showing the fluorescence of each of the samples for repeat two, 2 g/L rhodamine B, *Myrmica rubra*

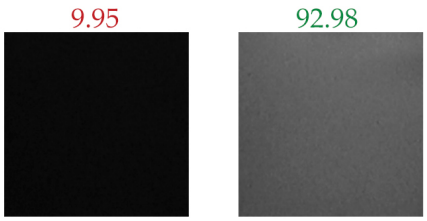


Figure F.30: Images showing the fluorescence of each of the samples for repeat three, 2 g/L rhodamine B, *Myrmica rubra*

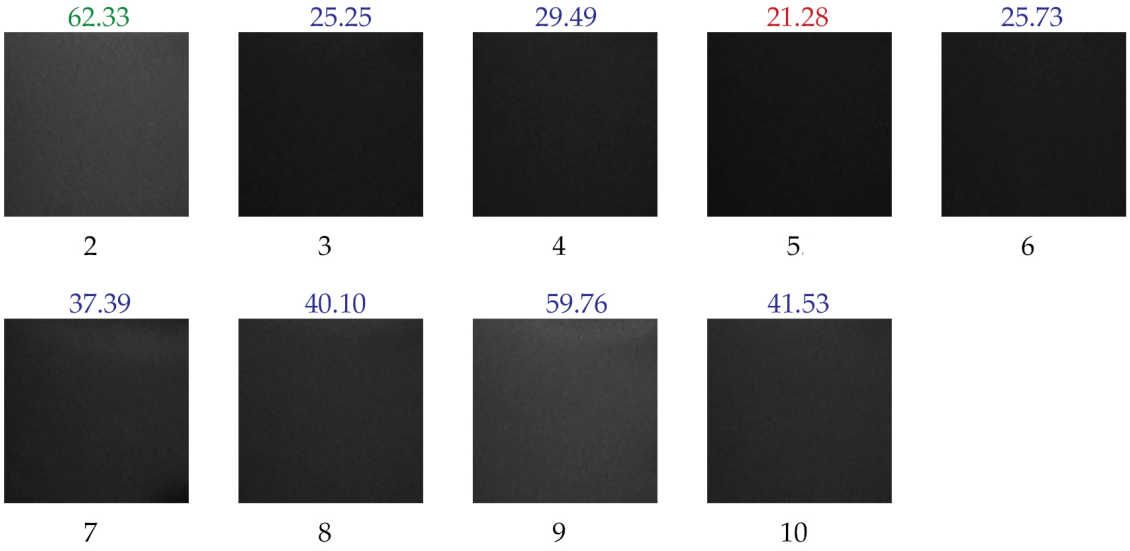


Figure F.31: Images showing the fluorescence of each of the samples for repeat one, 5 g/L rhodamine B, *Myrmica rubra*

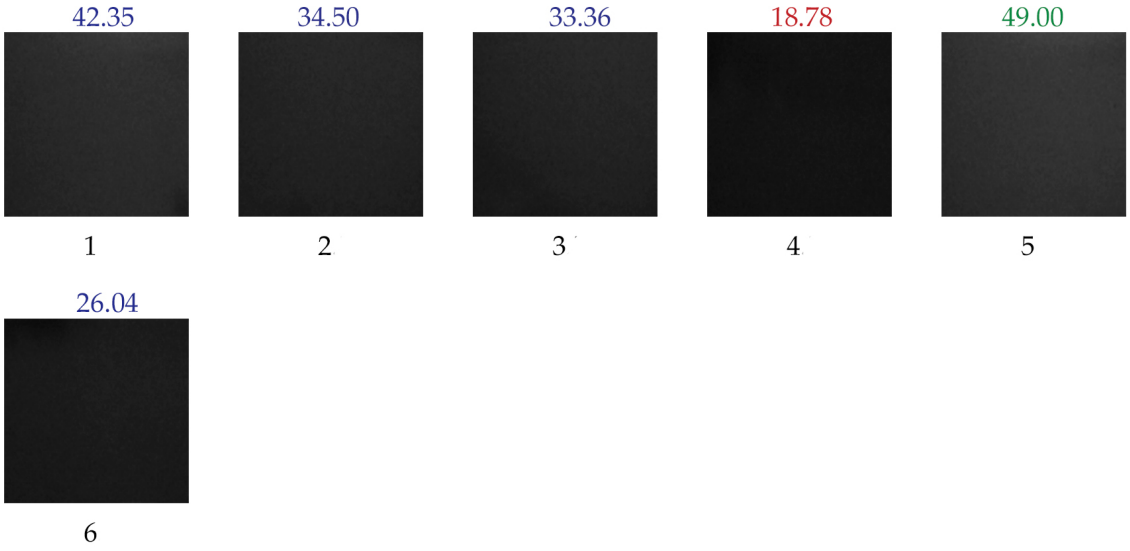


Figure F.32: Images showing the fluorescence of each of the samples for repeat two, 5 g/L rhodamine B, *Myrmica rubra*

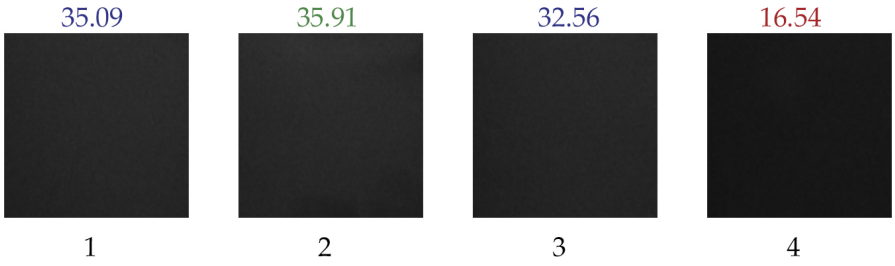


Figure F.33: Images showing the fluorescence of each of the samples for repeat three, 5 g/L rhodamine B, *Myrmica rubra*

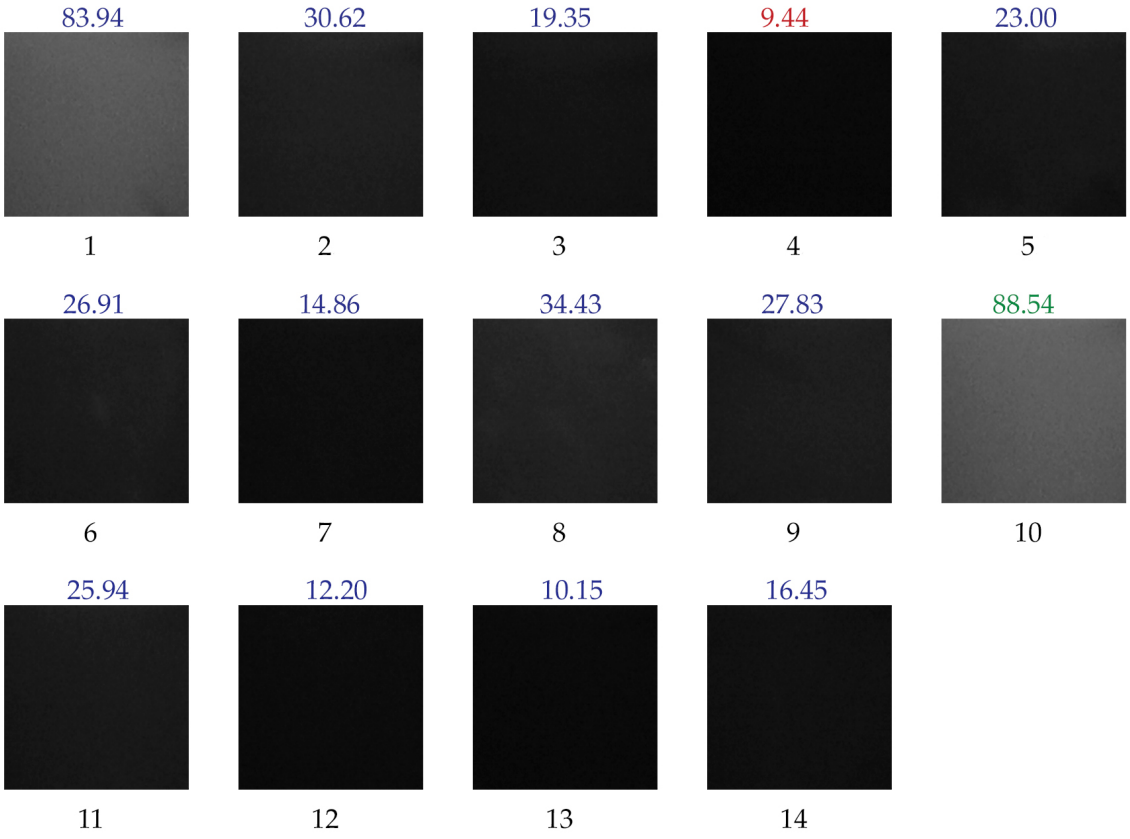


Figure F.34: Images showing the fluorescence of each of the samples for the basic yellow dye only control for *Myrmica rubra*

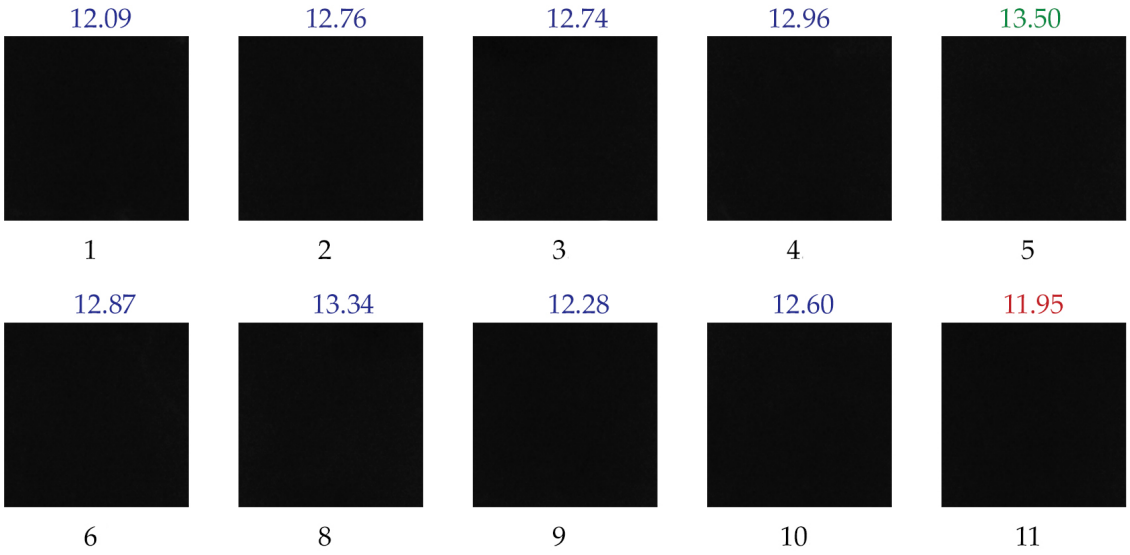


Figure F.35: Images showing the fluorescence of each of the samples for the sodium [$^{13}C_2$]acetate control for *Myrmica rubra* when imaged using a blue-green excitation source and an orange filter